Application Note

Clinical & Diagnostic

[^{18}F]FDG - Fluorodeoxyglucose according to USP

- **Analysis of FDG and its by-products (CDG & FDM)**
- **New USP L46 compatible stationary phase**
- **Fast high-resolution separation < 9 min**
- **U.S. Pharmacopeia 46 - NF41 (2023)**

The most selective LC-EC applications for Clinical & Diagnostics analysis

Catecholamines
Serotonin
Metanephrines
VMA
HVA
5-HIAA

PET imaging tracer
Fluorodeoxyglucose (FDG)
FDG impurities

Sulfides
Homocysteine
Glutathione
Disulfides

Vitamins, minerals
A, C, D, E, and K
Iodide
Q10
Ubiquinols

Summary

The new Antec Scientific SweetSep^{TM} AEX20 column, packed with a novel USP L46 compatible stationary phase based on highly monodisperse 5 µm resin particles, was evaluated for FDG impurity analysis according to the U.S. Pharmacopeia. This method to determine the purity of 2-deoxy-2-[^{18}F] fluoro-D-glucose (FDG) is based on High Performance Anion-Exchange Chromatography in combination with Pulsed Amperometric Detection (HPAEC-PAD). The column performance was tested in combination with the ALEXYS carbohydrates analyzer using the HPAEC-PAD method and conditions outlined in the section ' limit 2-chloro-2-deoxy-D-glucose' (FDG related compound B) in the official USP-NF 2023 monograph [1].

The new AEX20 4 x 200 mm anion-exchange column enables fast high-resolution analysis of FDG and its by-products 2-chloro-2-deoxy-D-glucose (CDG) and 2-fluoro-2-deoxy-D-mannose (FDM), resulting in the elution of all compounds of interest within 9 minutes with superior resolution compared to traditional USP L46 phases listed in the USP chromatographic column database [2]. Utilizing the AEX20 column resulted in an significant method improvement in terms of analysis time, resolution ($Rs_{(FDM-FDG)}$ & $Rs_{(FDG-CDG)}$) and sensitivity (S/N ratio). For operators involved in radio pharmaceutical Quality Control these improvements may contribute to a more robust and hassle-free impurity analysis of FDG following the USP guidelines at their hospital.

[^{18}F]FDG - Fluorodeoxyglucose according USP

Introduction

Fluorodeoxyglucose, [^{18}F]-FDG (abbreviated as FDG), is a radio-pharmaceutical widely used in positron emission tomography (PET) imaging, primarily for cancer diagnosis, treatment monitoring, and research. FDG is a glucose analogue, when administered to patients, allows for the visualization of metabolic activity within tissues. Cancer cells, which typically exhibit elevated rates of glucose metabolism compared to normal tissues, show increased uptake of FDG, making it a powerful tool for identifying and assessing tumors. Additionally, FDG PET scans provide valuable insights into various neurological and cardiac conditions, thus enhancing the understanding and management of a range of diseases. Its efficacy and non-invasive nature have made FDG one of the most utilized agents in nuclear medicine today [3].

FDG can be synthesized via different routes using fluorine-18 produced in a cyclotron [4]. The epimer 2-fluoro-2-deoxy-D-mannose (FDM) may be formed as a by-product in FDG production, depending on the synthesis route used [5]. 2-Deoxy-2-chloro-D-glucose (CDG) is an FDG-related impurity which can be formed by displacement of chloride with fluoride on carbon-2(C-2) during the nucleophilic fluorination step or during the hydrolysis step when HCl is used to remove the protective acetyl groups [4]. Therefore, a chemical purity analysis is one of the QC tests that must be conducted on the FDG solution before it can be injected into a patient. HPAEC-PAD is the method of choice for sensitive detection of CDG and FDM in FDG preparations [6,7]. A compendial method for the analysis of FDG-related compound B (CDG) is described in the U.S Pharmacopeia (USP) for FDG preparations in which CDG is the potential process impurity [1].

Figure 1. ALEXYS Carbohydrate Analyzer consisting of the ET 210 eluent tray (for N2 blanketing), a P 6.1L quaternary LPG pump, AS 6.1L autosampler, CT 2.1 column thermostat, and the DECADE Elite electrochemical detector.

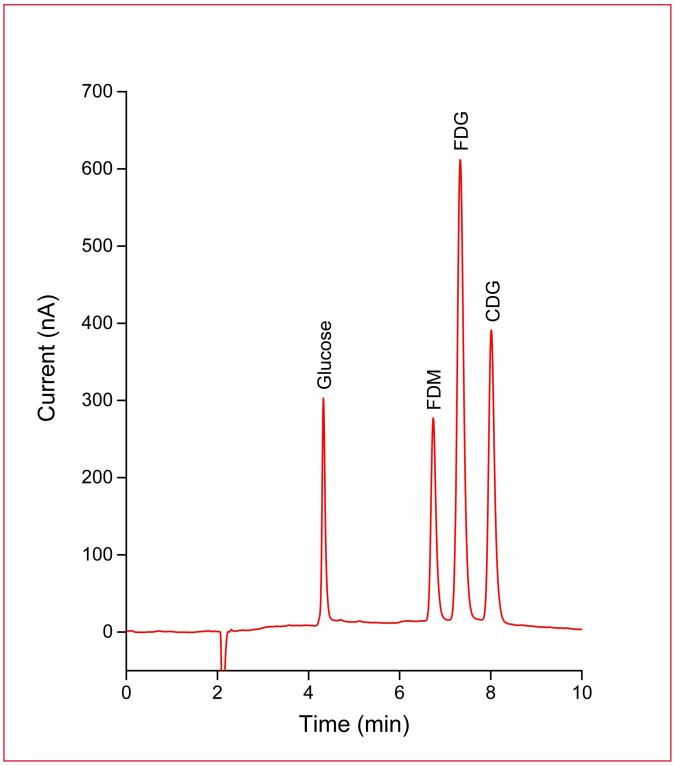

Figure 2. Chromatogram obtained from a standard mix consisting of 10 µg/mL FDG, FDM, CDG and 1 µg/mL glucose in water (10 µL injection). Resolution between FDM-FDG and FDG-CDG are 2.5 and 2.7, respectively.

In this application note data are presented of the impurity analysis of FDG using the new SweetSep™ AEX20 column, an USP L46 compatible stationary phase based on 5 µm polymer particle technology.

Table 1

LC-ECD conditions	
HPLC	ALEXYS Carbohydrate Analyzer
Columns	SweetSep™ AEX20, 4 × 200 mm column, 5 µm Borate ion trap, 4 x 50 mm column, 10 µm (Antec Scientific)
Filter	Pre-column filter PEEK, 0.5 µm
Mobile phase	100 mM NaOH prepared and blanketed with Nitrogen 5.0 gas
Flow rate	0.4 mL/min
System backpressure	About 100 bar
Temperature	35°C for separation and detection
Injection volume	10 µL
Pump piston wash	DI water (refresh weekly)
Flow cell	SenCell™ with 2 mm Au and HyREF (Pd/H_2), 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	0.1 Hz
I-cell	About 0.4 - 0.6 µA

Method

The U.S. Pharmacopoeia method to quantify FDG-related compound B (CDG) in 'hot' ^{18}F-FDG preparations is based on isocratic separation on a suitable anion exchange column using an alkaline mobile phase (pH 13), followed by pulsed amperometric detection on a gold (Au) working electrode. The conditions used for the impurity analysis are summarized in Table 1 and the method is validated according to the system suitability criteria as listed by the USP: resolution, tailing factor and signal-to-noise ratio.

For the method evaluation the ALEXYS carbohydrate analyzer was used (Fig 1.). The ALEXYS carbohydrate analyzer is a dedicated system for sensitive analysis of carbohydrates using HPAEC-PAD. The system is equipped with an ET 210 eluent tray, quaternary P 6.1L LPG pump, AS 6.1L auto sampler, CT 2.1 column thermostat and DECADE Elite electrochemical detector with SenCell. The ET 210 eluent tray enables to blanket the mobile phases with an inert gas atmosphere using special pressure-resistant plastic bottles made of polypropylene copolymer (PPCO). The inert atmosphere in the mobile phase bottle prevents diffusion of air into the mobile phase and will keep it free of CO_2 and O_2. Especially in carbohydrate analysis using HPAEC-PAD with strong alkaline eluents, dissolved CO_2 can be problematic. At pH > 12, CO_3^{2-} ions can be formed in the mobile phase, causing variations in retention times, decrease in column selectivity and loss in resolution.

In case the number of samples per day is limited, the ALEXYS carbohydrates analyzer can also be configured with a manual injection valve instead of an autosampler (see ordering information on the last page).

Separation

The mobile phase of 100 mM NaOH (pH 13) was carefully prepared manually using a carbonate-free 50% w/w NaOH solution (commercially available). The diluent was deionized water (resistivity 18.2 MΩ.cm, TOC < 5 ppb) which was sonicated and sparged with nitrogen 5.0 (purity >99.999%) prior to use. The mobile phase was prepared in a PPCO bottle supplied with the ALEXYS carbohydrate analyzer. 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. The appropriate amount of NaOH solution was carefully pipetted into the diluent under gently stirring and nitrogen sparging. After stirring the bottle was closed and the headspace above the mobile phase was blanketed with nitrogen 5.0 (0.2 – 0.4 bar overpressure) during analysis.

Column

The USP FDG monograph describes the following columns for the analysis of FDG:

Guard: 4 mm x 5 cm; 10 µm packing L46
Analytical: 4 mm x 25 cm; 10 µm packing L46

the USP L46 stationary phase being defined as a polystyrene/ divinylbenzene substrate agglomerated with quaternary amine functionalized latex beads, about 9 µm to 11 µm in diameter [2]. Antec Scientific has introduced a novel anion-exchange stationary phase, AEX20, which can be considered as an USP L46 compatible stationary phase. The AEX20 resin consist of highly monodisperse 5 µm polystyrene/divinylbenzene particles agglomerated with latex nano beads functionalized with quaternary amine groups. The smaller particle size of 5 µm of the AEX20 resin enables fast high-resolution separation of FDG and its side products.

An 4 x 200 mm AEX20 analytical column without guard column was used for this evaluation to reduce the total analysis time and ensure compliance with the maximum allowed adjustments of chromatographic parameters as described in USP general chapter <621>, see table 2 [8]. A 0.5 µL PEEK precolumn filter with 0.5 µm pore size was used to prevent the column from clogging with particular matter.

Column dimensions: The USP general chromatography chapter <621> allows adjustments of the column length (L) and particle size (dp) as long as the L/dp ratio remains constant or in the range of –25% to + 50% of the column specified in the monograph.

Table 2

Allowed adjustments of chromatographic parameters following USP general chapter <621> (isocratic elution)

Adjustment	*USP*	*Description*
Column dimensions (L/dp ratio)	-25% - +50%	Particle size and/or length may be modified providing that the ratio column length/particle size (L/dp ratio) remains constant or in the range between -25% to +50%.
Flow rate	± 50%	Must be adjusted if column diameter or particle size has been changed using the equation "F_2"described in reference [9]. After adjustment due to a change in column dimensions an additional change in flow rate of ± 50% is permitted.
Injection volume	The injection volume might be varied providing system requirements remain within their established acceptability limits.	In case column dimensions are changed injection volume may be adjusted using the equation "V_{inj2}" described in ref [9]. Injection volume may be decreased, providing that detection and repeatability remain satisfactory. Injection volume may be increased providing that resolution and linearity of peaks remain satisfactory.

In this case the prescribed L/dp ratio is 30000, based on the length of the analytical + guard column (300 mm) and 10 µm particle size. The L/dp ratio based on the 200 mm AEX20 column and 5 µm particle size is 40000. The difference in ratio of +33% falls within the specified upper limit of +50%.

Flow rate: the USP outlines the following equation for the adjustment of flow rate in case columns with another internal diameter and/or particle size are used:

$F_2 = F_1 \times [dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)]$

Where F_1 and F_2 are the flow rates for the original and modified conditions, respectively; dc_1 and dc_2 are the respective column diameters, and dp_1 and dp_2 the particle sizes. In addition, after adjustment due to a change in column dimensions an additional change in flow rate of ± 50% is permitted.

The corrected flow rate based on the change in particle size, column length and inner diameter was calculated using the before mentioned formula. To compensate for the applied adjustments a flow rate of 0.8 mL/min was calculated. However, the original flow rate of 0.4 mL/min (-50%) mentioned in the monograph was chosen for the AEX20 column as well. This flow rate is optimal for the specific column in terms of resolution, minimizes eluent consumption, and remains within the allowed flow rate adjustment limit (± 50%) outlined in the USP general chapter <621>.

Injection volume: the USP general chapter states that the following equation may be used for adjustment of the injection volume when the column dimensions are changed:

$V_{inj2} = V_{inj1} \times [(L_2 \times dc_2^2)/(L_1 \times dc_1^2)]$

Where, V_{inj1} and V_{inj2} are the injection volumes for the original and modified conditions, respectively; L_1 and L_2 are the respective column lengths, and dc_1 and dc_2 the corresponding internal diameters. However, regardless if the column dimension are changed or remain the same, the injection volume may be increased providing that resolution and linearity of peaks remain satisfactory.

The corrected injection volume V_{inj2} calculated using the before -mentioned equation is 6.7 µL. Nonetheless, an injection volume of 10 µL as specified in the monograph was used to maximize S/N ratio, while maintaining excellent peak resolution and linearity.

A borate ion trap column (4 x 50 mm) was installed in the solvent line between the pump and autosampler as precaution to eliminate borate ions from the mobile phase [9].

Detection

For the detection of FDG and its by-products, the Antec SenCell™ electrochemical flow cell is used [10]. This user-friendly flow cell with wall-jet design consists of a Au working electrode, maintenance-free palladium hydrogen (HyREF) reference electrode, and stainless steel auxiliary electrode. In the USP monograph no specific potential waveform for detection is described, therefore we applied the 4-step potential waveform optimized for carbohydrate detection. This particular waveform resulted in an excellent reproducibility and minimal electrode wear [11]; i.e. resulting in less flow cell maintenance and system down time. The cell current was typical about 0.5 µA under the specified conditions.

Temperature

In the section of the FDG monograph addressing the 'limit of FDG-related compound B' no temperature settings are specified. Therefore, a temperature for separation and detection was selected of 35°C for optimal performance with respect to peak resolution and S/N ratio.

Results

System suitability

In the USP monograph for [^{18}F]FDG injections the following system suitability requirements are specified to evaluate the system performance: resolution, tailing factor and signal-to-noise ratio. A chromatogram of a 10 µL injection of the system suitability solution is shown in Figure 3. This system suitability solution consists of a mix of 0.01 mg/mL USP Fludeoxyglucose RS (FDG) and USP Fludeoxyglucose Related Compound B RS (CDG) in DI water and is used to determine the resolution

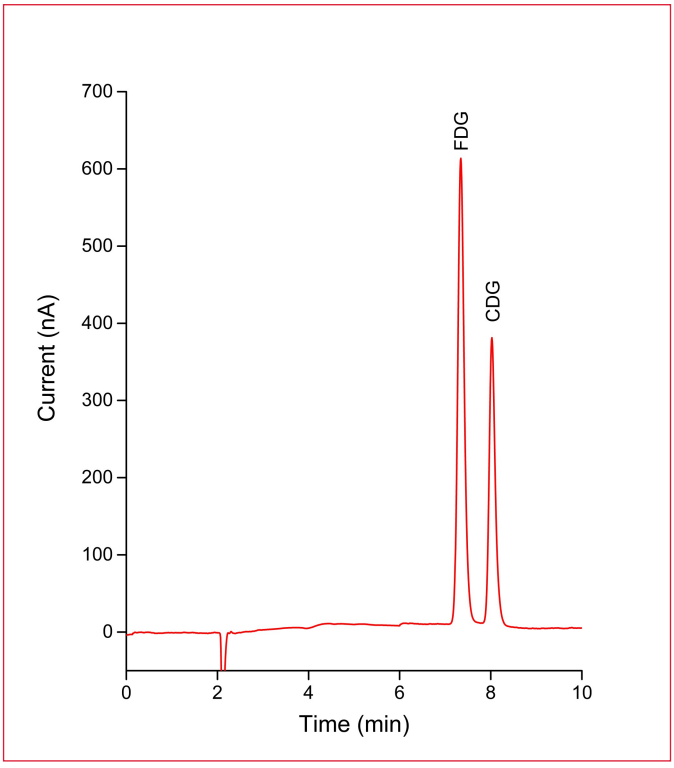

Figure 3. Chromatogram obtained with a 10 µL injection of the system suitability solution consisting of 10 µg/mL CDG and FDG in DI water.

between FDG and CDG. To determine the tailing factor and signal-to-noise ratio, the USP standard solution consisting of 0.01 mg/mL USP Fludeoxyglucose Related Compound B RS in DI water was used. The results of the system suitability test are listed in Table 2. it is evident that the system performance parameters obtained with the ALEXYS carbohydrate analyzer in combination with the AEX20 column are well within the SST criteria of the USP.

Table 3

USP system suitability requirements

Parameter	*USP criteria*	*Measured*
Resolution between FDG and CDG	> 1.0	2.7
Tailing factor (CDG)	< 1.5	1.2
Signal-to-noise ratio (CDG)	> 50	1100

Linearity, repeatability, and LOD

The linearity for CDG, FDG and FDM was investigated in the concentration range of 1 – 50 µg/mL and between 0.1 - 5 µg/mL for glucose , see Table 4. The linear correlation coefficient was > 0.9999 for all four analytes.

Table 4

Linearity

Compound	*Concentration range (µg/mL)*	*R*
Glucose	0.1 - 5	0.99999
FDM	1 - 50	0.99997
FDG	1 - 50	0.99998
CDG	1 - 50	0.99999

The relative standard deviation (RSD) in peak area was determined for 10 replicate injections of the 10 µg/mL FDM, FDG and CDG standard mixture solution including 1 µg/mL glucose (see figure 1). The RSD in peak area was about 1% which together with a RSD retention time of 0.1% demonstrates the excellent repeatability of the method (see table 5).

Table 5

Repeatability

Compound	*RSD t_R (%)*	*RSD Area (%)*
Glucose	0.10	0.94
FDM	0.08	1.04
FDG	0.07	0.99
CDG	0.09	0.80

The Limit of Detection (LOD) of the method was determined based on the responses obtained with a 10 µL injection of a standard mix containing 10 ng/mL glucose and 100 ng/mL FDM, FDG and CDG in DI water. The calculated LOD values are listed in Table 6.

Table 6

Limit of Detection (LOD)

	LOD (ng/mL)	*LOD (nM)*	*On-column (pg)*
Glucose	3	14	26
FDM	30	167	304
FDG	17	96	174
CDG	22	113	224

The chromatogram run time was extended to 20 minutes to be able to determine the baseline noise. The ASTM noise was determined over a 10 minute section of the baseline (t = 10 min to 20 min) using the average peak-to-peak noise of 20 segments of 0.5 min. The LODs were calculated as the analyte response corresponding to 3× the ASTM noise. The high sensitivity of the method is evident from the LOD reported for Glucose. This level of sensitivity is typically achievable using the ALEXYS carbohydrate analyzer under isocratic separation conditions in HPAEC-PAD analysis. The response factors of the halogen-substituted glucose derivatives are a factor 7 - 12 lower than glucose.

Limit of FDG-related compound B & LOQ

The USP acceptance criteria for the maximum amount of CDG allowed in ^{18}F-FDG injections is 1.0 mg/V, where V is the volume (mL) of the dose injected [1]. The ^{18}F-FDG dose is typically calculated based on activity per unit body weight, expressed in megabecquerels per kilogram (MBq/kg) or millicuries per kilogram (mCi/kg). The radiopharmaceutical is often prepared as a solution with a specific concentration (e.g., MBq/mL). The injection volume depends on this concentration and the required activity. Typically, the volume does not exceed 10 mL to minimize patient discomfort and avoid overloading the injection site [12]. Taking into account a maximum dose volume of 10 mL, the limit of 1.0 mg/V corresponds to a maximum concentration of 0.1 mg/mL CDG in the ^{18}F-FDG sample. The LOQ for CDG of the method was determined as 60 ng/mL, which is a factor 1500+ lower than the limit. It is evident that which such LOQ, concentrations of CDG in the sample around the USP limit, can be determined with excellent accuracy.

Sample analysis

The primary method for routine production of ^{18}F-FDG injectables involves using saline combined with a small residual amount of ethanol as stabilizer, along with a phosphate or citrate buffer during the synthesis process. The USP monograph requires the injection of undiluted ^{18}F-FDG saline solution for the quantification of the impurity level of CDG in the sample. To assess if the saline matrix affects the chromatography and thus peak performance parameters, a FDG standard solution was prepared in a saline matrix in combination with a citrate buffer. The matrix composition used for the FDG solution was based on information received from a customer involved in ^{18}F-FDG production and QC: 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. The chromatogram obtained with a 10 µL injection of 1 µg/mL glucose and 10 µg/mL CDG, FDG and FDM in a saline - citrate buffer matrix is shown in figure 4.

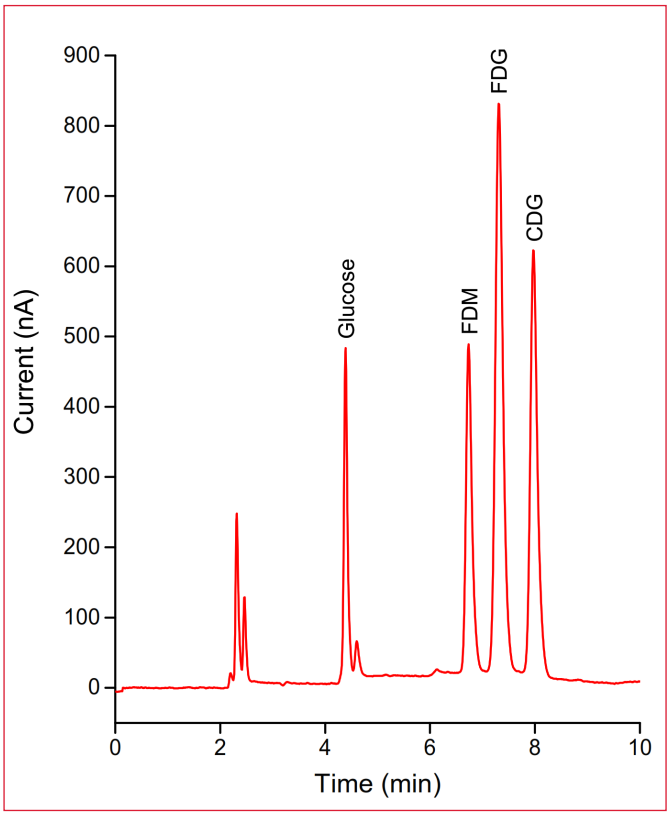

Figure 4. Chromatogram obtained with a 10 µL injection of 1 µg/mL glucose and 10 µg/mL CDG, FDG and FDM in a saline - citrate buffer matrix.

In table 7 the peak performance data obtained from the FDG solution in saline – citrate buffer matrix are listed. All values (resolution, tailing factor and SN ratio) are well within the USP system suitability requirements and demonstrate that the AEX20 column is a perfect choice for the impurity analysis of CDG in ^{18}F-FDG injectables following USP.

Table 7

Performance data, FDG in saline - citrate buffer matrix

Parameter	*USP criteria*	*Measured*
Resolution between FDG and CDG	> 1.0	2.5
Tailing factor (CDG)	< 1.5	1.3
Signal-to-noise ratio (CDG)	> 50	831

Precolumn

For operators who want to perform the impurity analysis with a guard column upfront the 4 x 200 mm AEX20 analytical column, we have a 4 x 50 mm AEX20 precolumn available. Although, in this case the formal USP 'L/dp' criteria for allowed changes in column dimensions are not met [8], it is evident that the separation performance improved with respect to resolution between FDG and CDG due to the increase in column length, see figure 5 and table 8.

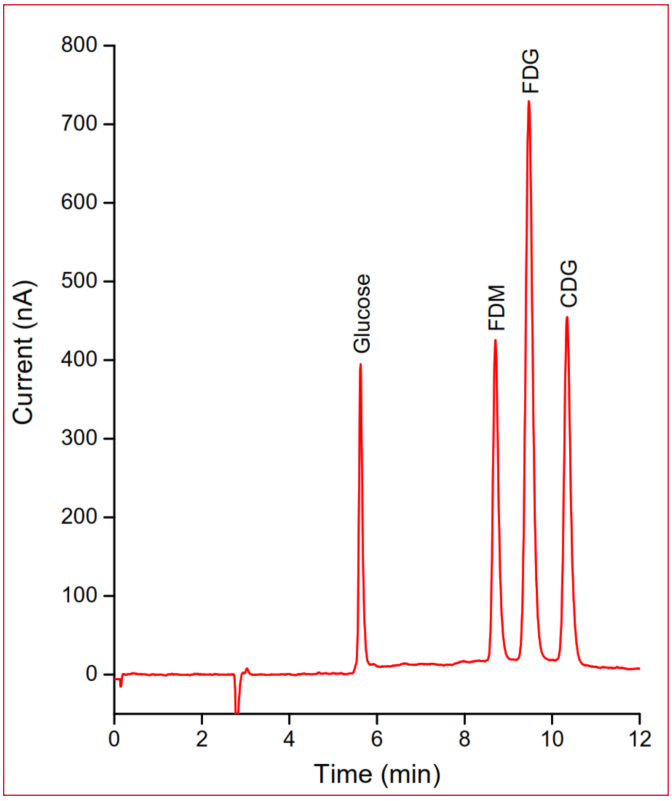

Figure 5. Chromatogram obtained with a 10 µL injection of 1 µg/mL glucose and 10 µg/mL CDG, FDG and FDM in DI water using a 4 x 50 mm AEX20 pre-column and 4 x 200 mm AEX20 analytical column in series.

Table 8

Performance data, FDG in saline - citrate buffer matrix

Parameter	*USP criteria*	*Measured*
Resolution between FDG and CDG	> 1.0	3.0
Tailing factor (CDG)	< 1.5	1.2
Signal-to-noise ratio (CDG)	> 50	885

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Conclusion

The ALEXYS carbohydrate analyzer in combination with the new SweetSep™ AEX20 column provides a reliable solution for fast & sensitive analysis of low level FDG impurities in commercial Fludeoxyglucose F 18 Injection samples based on the official USP method. The use of the new SweetSep™ AEX20, as USP L46 compatible alternative, resulted in separations with an exceptionally good resolution between FDG, FDM and CDG. In combination with the high sensitivity of the ALEXYS Carbohydrate Analyzer, this excellent performance ensures that the system suitability requirements are easily met and may contribute to more robust and hassle-free impurity analysis.

[^{18}F]FDG - Fluorodeoxyglucose according USP

Ordering information

Detector only	
176.0035B*	DECADE Elite SCC electrochemical detector
116.4321	SenCell 2 mm Au HyREF
ALEXYS FDG analyzer (manual injector)	
180.0053WM	ALEXYS FDG Analyzer (incl. SenCell & Clarity CDS software#)
ALEXYS analyzer (AS 6.1L autosampler)	
180.0055W	ALEXYS Carbohydrates Analyzer - isocratic
116.4321	SenCell 2 mm Au HyREF
195.0035#	Clarity CDS single instr. incl LC, AS module
Columns	
260.0020	SweetSep™ AEX20, 4 x 200 mm column, 5 µm
260.0025†	SweetSep™ AEX20, 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

*) For the DECADE Elite electrochemical detector control drivers are available in DataApex Clarity CDS, Thermo Fisher Scientific Chromeleon™ CDS, Waters Empower™, Agilent OpenLab CDS and Agilent OpenLab CDS Chemstation Edition. Please contact Antec for more details.

#) The ALEXYS Carbohydrates Analyzer and ALEXYS FDG analyzer can also be fully controlled under Thermo Fisher Scientific Chromeleon™ CDS. Please contact Antec for more details.

†) Optional in case a precolumn is preferred, see last paragraph of the note for important information about allowed changes following the USP <621> general chromatography chapter [8] .

**)The pre-column filter PEEK, 0.5 µm (pn 260.0100) includes a starter pack of 4 replacement PEEK frits. A 10 pack of replacement PEEK frits can be ordered under pn 260.0110.

Figure 6. 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 acquisition.

For research purpose only. 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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