

Application Note

Clinical & Diagnostic



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 lodide Q10 Ubiquinols

[¹⁸F]FDG - Fluorodeoxyglucose according to EP method



- Analysis of FDG and its by-products (CDG & FDM)
- SweetSepTM AEX18 (USP L46 column)
- Fast high-resolution separation < 5 min
- European Pharmacopeia 11.3 (2024)
- 'Green' analytical method

Summary

The new Antec Scientific SweetSep[™] AEX18 column, packed with a novel USP L46 listed stationary phase based on highly monodisperse 5 µm resin particles, was evaluated for FDG impurity analysis according to the European Pharmacopeia (EP). The EP method to determine the purity of 2-deoxy-2-[¹⁸F]fluoro-D-glucose (FDG) is based on High Performance Anion-Exchange Chromatography in combination with Pulsed Amperometric Detection (HPAEC-PAD). The column performance was evaluated in combination with the ALEXYS carbohydrates analyzer using the HPAEC-PAD method and conditions outlined in the section '2-Fluoro-2-deoxy-D-glucose and impurity A' in the official EP 11.3 monograph for FDG [1].

The new AEX18 2.1 x 185 mm anion-exchange column enables fast high-resolution analysis of FDG and its by-products 2-chloro-2-deoxy-D-glucose (CDG, impurity A) and 2-fluoro-2-deoxy-D-mannose (FDM), resulting in the elution of all compounds of interest within 5 minutes with superior resolution compared to traditional anion-exchange resins based on 10 µm particles [2]. Utilizing the AEX18 column resulted in an significant method improvement in terms of analysis time, resolution (Rs_(FDM-FDG) & Rs_(FDG-CDG)) and sensitivity (S/N ratio). In addition, the use of a 2.1 mm ID narrow-bore column minimizes solvent consumption and waste, thus reducing environmental impact. For operators involved in radio pharmaceutical Quality Control these improvements will contribute to a more robust and hassle-free impurity analysis of FDG following the EP guidelines at their hospital.

Electrochemistry Discover the difference



Introduction

Fluorodeoxyglucose, [¹⁸F]-FDG (abbreviated as FDG), is a radiopharmaceutical 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-Dmannose (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 impurity A (CDG) is described in the European Pharmacopeia (EP) 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 N_2 blanketing), a P 6.1L quaternary LPG pump, AS 6.1L autosampler 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 DI water (2 µL injection). Resolution between FDM-FDG and FDG-CDG are 2.4 and 2.5, respectively.

In this application note data are presented of the impurity analysis of FDG following EP 11.3 using the new SweetSep[™] AEX18 column, an USP L46 listed stationary phase based on 5 μm polymer particle technology.

Table 1

LC-ECD conditions

HPLC	ALEXYS Carbohydrate 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	Pre-column filter PEEK, 0.5 μm	
Mobile phase	90 mM NaOH, prepared by LPG mixing of (A) 100 mM	
	NaOH and (B) DI water, in a proportion ratio of A%:B% =	
	90 : 10. All solutions blanketed with nitrogen 5.0	
Flow rate	0.28 mL/min	
System backpressure	About 191 bar	
Temperature	35°C for separation and detection	
Injection volume	2 μL (full loop)	
Pump piston wash	DI water (refresh weekly)	
Flow cell	SenCell [™] with 2 mm Au WE and HyREF Pd RE.,	
	AST pos. 2	
Potential waveform	E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V	
(4-step)	ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s	
Range	2 μΑ/V	
ADF	0.5 Hz	
I-cell	About 0.2 - 0.4 μA	



Method

The European Pharmacopoeia method to quantify FDG impurity A (CDG) in 'hot' ¹⁸F-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 EP: resolution and signal-to-noise ratio.

For the method evaluation the ALEXYS carbohydrate analyzer was used (Fig 1). The ALEXYS carbohydrate analyzer is a dedicated metal-free IC 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 and DECADE Elite electrochemical detector with SenCell. The ET210 eluent tray has an integrated gas distribution system to blanket the headspace of the eluent bottles with inert gas (Nitrogen or Helium), to prevent CO₂ uptake from the surrounding atmosphere and the build up of carbonate ions $(CO_3^{2^c})$. In HPAEC-PAD analysis dissolved carbonate ions can be problematic, because these anions act as a strong 'pushing' agent causing a loss in retention and resolution over time.

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 and Figure 5 on the last page).

Column

The EP FDG monograph describes the following stationary phase for the analysis of FDG: a strongly basic anion-exchange resin for chromatography R ($10 \mu m$) with a length of 0.25 m and diameter of 4.0 mm. According to the EP chapter 4.1.1. Reagents [8], this is a resin with quaternary amine groups attached to a lattice of latex cross linked with divinylbenzene.

Antec Scientific has introduced a novel anion-exchange stationary phase, AEX18, which is a USP L46 listed stationary phase consisting of highly monodisperse 5 μ m polystyrene/ divinylbenzene particles agglomerated with latex nano beads functionalized with quaternary amine exchange groups. The smaller particle size of 5 μ m of the AEX18 resin enables fast high-resolution separation of FDG and its side products. For this evaluation an 2.1 x 185 mm AEX18 microbore column was used in combination with 0.5 μ L PEEK precolumn filter with 0.5 μ m pore size, to prevent the column from clogging with particular matter. In HPAEC-PAD carbohydrate analysis, low μ g/L borate eluent contaminations can harm chromatographic efficiency due to complexation reactions with borate. To prevent this, a borate ion trap column was installed between the pump and injector [10].

The EP general chapter 2.2.46 'Chromatographic separation techniques', describes the allowed adjustments of chromatographic parameters which can be applied to optimize the separation of an LC method in an EP monograph [9]. In Table 2 a selection of the allowed adjustments are listed, which are optimized to improve the analysis of FDG impurity A using HPAEC-PAD.

<u>Column internal diameter:</u> there are no restrictions on the adjustment of the column internal diameter. The internal diameter of the column may be adjusted, even in the absence of a change in particle size and/or length of the column. A column ID of 2.1 mm was chosen (instead of 4 mm) to lower mobile phase consumption and increase mass sensitivity.

Table 2

Allowed adjustments of chromatographic parameters following EP 11.6 general chapter 2.2.46 (isocratic elution)

Adjustment	EP	Description
Mobile phase composition & pH	± 10% pH: ± 0.2 pH units	No component may be altered by more than 10 percent absolute. The pH of the aqueous component of the mobile phase may be altered \pm 0.2 pH units, unless otherwise prescribed.
Flow rate	50%	Flow rate must be adjusted for changes in column diameter and particle size using the flow rate equation as described in ref [9]. After an adjustment due to a change in column dimensions an additional change in flow rate of ± 50% is permitted.
Particle size & column length (L/dp ratio)	-25% - +50%	The particle size and/or length of the column may be modified providing that the ratio of the column length L) to the particle size (dp) remains constant in the range of -25% - +50% of the prescribed L/dp ratio
Internal diameter	No restrictions	The internal diameter of the column may be adjusted, even in the absence of a change in particle size and/or length of the column.
Temperature	± 10°C	10°C deviation allowed where the operating temperature is specified, unless otherwise prescribed.
Injection volume	Decrease allowed	In case column dimensions are changed injection volume may be adjusted using the equation described in ref [9]. Even in the absence of any column dimension change the Injection volume may be decreased, providing that detection and repeatability remain satisfactory. Injection volume may be increased providing that resolution and linearity of peak(s) remain satisfactory.



<u>Column dimensions (particle size, length)</u>: The EP 11.6 general chapter 2.2.46 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. The L/dp ratio of the column prescribed in the FDG monograph is 25000 (length 250 mm, particle size 10 μ m). The SweetSep AEX18 column with a length of 185 mm and 5 μ m particle size has a L/dp ratio of 40000. The difference in ratio of +48% falls within the specified upper limit of +50%.

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

$$F_{2} = F_{1} \times [dc_{2}^{2} \times dp_{1})/(dc_{1}^{2} \times dp_{2})]$$
(1)

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 \pm 50% is permitted by the EP. The corrected flow rate based on the change in particle size and inner diameter using eq. 1 is 0.55 mL/min. However, a lower flow rate of 0.28 mL/min (-50%) was chosen for the analysis. This flow rate is optimal for the AEX18 column in terms of resolution, minimizes eluent consumption, and remains within the allowed flow rate adjustment limit (\pm 50%) outlined in the EP general chapter.

<u>Temperature</u>: the separation should be performed at 25°C according to the EP monograph, but an adjustment of ±10°C is allowed. A temperature of 35°C was selected for both separation and detection, because under this condition excellent separation and detection (peak resolution and S/N ratio) was achieved in combination with a shorter run time and lower back pressure.

<u>Injection volume</u>: the injection volume specified in the monograph is 20 μ L based on a 4 mm ID column. the EP general chapter states that the following equation may be used for adjustment of the injection volume when the column dimensions are changed:

$$V_{inj2} = V_{inj1} \times [(L_2 \times dc_2^2)/(L_1 \times dc_1^2)]$$
(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. Regardless if the column dimension are changed or remain the same, the injection volume may be decreased providing that S/N ratio and repeatability remain satisfactory. The corrected injection volume V_{inj2} calculated using the before-mentioned eq, 2 is 4.1 µL. Nonetheless, an smaller injection volume of 2 µL was selected to maximize peak resolution, while maintaining excellent S/N ratio and repeatability. Mobile phase: the monograph prescribes 100 mM NaOH as eluent for the separation of FDG and FDG impurity A. During this evaluation the separation was optimized taking into account the allowed changes in mobile phase composition described in the general chapter. The actual eluent used for the separation was 90 mM NaOH, prepared by LPG mixing of (A) 100 mM NaOH with (B) DI water, in a proportion ratio of A%:B% = 90 : 10.

Mobile phase A, 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 M Ω .cm, TOC < 5 ppb) which was sonicated and sparged with nitrogen 5.0 (purity \geq 99.999%) prior to use. The mobile phase was prepared in a polypropylene (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 during analysis (0.2 – 0.4 bar N₂ pressure relative to ambient).

Detection

For the detection of FDG and its by-products, the Antec SenCellTM electrochemical flow cell is used [11]. This userfriendly 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 EP monograph no specific potential waveform for detection is described, therefore we applied the classical 4-step potential waveform optimized for carbohydrate detection. This particular waveform resulted in an excellent reproducibility and minimal electrode wear [12]; i.e. resulting in less flow cell maintenance and system down time. The cell current was typical about 0.2-0.4 μ A under the specified detection conditions.

Results

System suitability

In the EP monograph 'Fludeoxyglucose [¹⁸F] injection' the following system suitability requirements are specified to evaluate the system performance: resolution and signal-tonoise ratio. A chromatogram of an 2 μ L injection of the system suitability solution is shown in Figure 3. This system suitability solution, reference solution c, consists of a mix of 25 μ g/mL FDM and 12.5 μ g/mL FDG (based on a maximum 20 mL dose) in DI water and is used to determine the resolution between FDM and FDG, and the signal-to-noise ratio of FDG.





Figure 3. Chromatogram obtained with a 2 μL injection of the system suitability solution (reference solution c) consisting of 25 μg/mL FDM and 12.5 μg/mL FDG* in DI water. FDG concentration in reference solution c based on a maximum recommended dose V of 20 mL.

Table 3

EP system suitability requirements

Parameter	EP criteria	Measured
Resolution between FDM and FDG	> 1.5	2.4
Signal-to-noise ratio (FDG)	> 10	1013

The results of the system suitability test are listed in Table 3. It is evident that the system performance parameters obtained with the ALEXYS carbohydrate analyzer in combination with the AEX18 column are well within the SST criteria of the EP.

Relative retention and total run time

The relative retention times (RRT) of the impurities with respect to FDG were determined using a chromatogram obtained from a 2 μ L injection of a standard solution containing 10 μ g/mL each of FDG, FDM, and CDG, and 1 μ g/mL of glucose in DI water. The RRT values are listed in Table 4. Compared to

Table 4

Retention time of FDG and related substances

Compound	Retention time (min)	Relative retention time*	Relative retention time EP
FDM	3.30	0.9	0.9
FDG	3.67	1.0	1.0
CDG	4.12	1.1	1.1

* Relative retention time with reference to FDG (3.67 min)

the relative retention times listed in the monograph, the results are identical. The EP specifies a retention time for FDG of about 12 minutes, and states that the total run time should be twice the retention time of FDG, so 24 minutes. FDG elutes with a retention time of 3.67 minutes with the improved method based on the AEX18 column, resulting in a total run time of only 7.4 minutes. So the presented method results in a more than 3x faster analysis time and thus a 3-fold increase of the sample throughput.

Linearity, repeatability, and LOD

The linearity for CDG, FDG and FDM was investigated in the concentration range of $1 - 50 \ \mu\text{g/mL}$ and between $0.1 - 5 \ \mu\text{g/}$ mL for glucose (6 calibration levels), see Table 5. The linearity is excellent with correlation coefficients of > 0.9999 for all four analytes.

Table 5

Linearity

Compound	Concentration range (µg/mL)	R
Glucose	0.1 - 5	0.99998
FDM	1-50	0.99995
FDG	1-50	0.99998
CDG	1-50	0.99997

The relative standard deviation (RSD) for peak area and retention time were determined for 10 consecutive injections of a standard solution containing 1 μ g/mL glucose and 10 μ g/ mL FDM, FDG and CDG in DI water. The results are presented in Table 6 and in Figure 1 an example chromatogram is shown. For all components the peak area RSD's were 0.2% or lower and the RSD's for retention time < 0.2%, demonstrating the good repeatability of the analysis of FDG and its impurities by this method.

Table 6

Repeatability (n=10)

Compound	RSD t _R (%)	RSD Area (%)
Glucose	0.18	0.21
FDM	0.09	0.10
FDG	0.09	0.07
CDG	0.08	0.07

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

Table 7

Limit of Detection (LOD) & Limit of Quantification (LOQ)			
Compound	LOD (nM)	LOD (ng/mL)	LOQ (ng/mL)
Glucose	35	6	21
FDM	380	69	231
FDG	190	34	115
CDG	241	48	159

The calculated LOD values are listed in Table 7. The ASTM noise was determined over a 3.5 minute section of the baseline (t = 3.5 min to 7 min) using the average peak-to-peak noise of 7 segments of 0.5 min. The LOD's and LOQ's were calculated as the analyte response corresponding to 3× and 10x the ASTM noise, respectively. The high sensitivity of the method is evident from the LOD reported for glucose. The response factors of the halogen-substituted glucose derivatives are 5 to 11 times lower than that of glucose.

Limit of FDG & impurity A

The EP acceptance criteria for the maximum amount of FDG and impurity A (CDG) allowed in 18 F-FDG injections is 0.5 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



Figure 4. Chromatogram obtained with a 2 μ L injection of 1 μ g/mL glucose and 10 μ g/mL CDG, FDG and FDM in a saline - citrate buffer matrix.

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. Taking into account a maximum recommended dose V of 20 mL, the limit of 0.5 mg/V corresponds to a maximum concentration of 25 μ g/mL FDG and CDG in the ¹⁸F-FDG sample. The LOQ for FDG of the method was determined as 115 ng/mL, which is more than 200 times lower than the limit. For FDG impurity A (CDG) the LOQ is more than 150 times lower than the limit. It is evident that with such LOQ's, concentrations of FDG and impurity A in the sample around the EP limit, can be determined with excellent accuracy.

Sample analysis

The primary method for routine production of ¹⁸F-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 EP monograph requires the injection of undiluted ¹⁸F-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 ¹⁸F-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. An example chromatogram obtained with an 2 μ L injection of 1 μ g/mL glucose and 10 μ g/ mL CDG, FDG and FDM in a saline - citrate buffer matrix is shown in Figure 4. The resolution between FDM-FDG and FDG-CDG are 2.4 and 2.5, respectively. These resolution values in saline-citrate buffer are identical to the values obtained with the FDG solution in DI water (Figure 1). The resolution of 2.4 between FDM and FDG meets the EP system suitability requirements and confirms that the AEX18 column is an excellent choice for impurity analysis of CDG in ¹⁸F-FDG injectables, in compliance with EP monograph.

Precolumn

For operators who want to perform the impurity analysis with a guard column upfront the 2.1 x 185 mm AEX18 analytical column for protection, we have a short 2.1 x 30 mm AEX18 precolumn available. Although, in this case the formal EP 'L/dp' criteria for allowed changes in column dimensions are not met [9], it is evident that the separation performance improved slightly with respect to resolution between FDG and CDG due to the increase in column length, see table 8 for SST results.



Table 8

Parameter	EP criteria	Measured
Resolution between FDM and FDG	> 1.5	2.5
Signal-to-noise ratio (FDG)	> 10	956

References

- 1. Fludeoxyglucose (¹⁸F) Injection, European Pharmacopeia 11.3 (2024), 5101-5103
- 2. Online EP Knowledge Database, <u>https://</u> <u>extranet.edqm.eu/4DLink1/4DCGI/Web_View/</u> <u>mono/1325</u>
- H.A. Nabi, J.M. Zubeldia, "Clinical Applications of 18F-FDG in Oncology", Journal of Nuclear Medicine Technology, Volume 30, Number 1, 2002, <u>https://</u> tech.snmjournals.org/content/jnmt/30/1/3.full.pdf
- S. Yu, "Review of ¹⁸F-FDG synthesis and quality control", Biomed Imaging Interv J, 2006; 2(4):e57, <u>http://www.biij.org/2006/4/e57</u>
- T.Ido et al., "Fluorination with F2, a convenient synthesis of 2-deoxy-2-fluoro-D-glucose". J. Org. Chem., Volume 42(13), 1977, 2341 <u>https://doi.org/10.1021/jo00433a037</u>
- D.L. Alexoff et al., "Ion Chromatographic analysis of high specific activity ¹⁸FDG preparations and detection of the chemical impurity 2-deoxy-2-chloro-D-Glucose", Appl. Radiat. Iot., Volume 43 (11), 1992, 1313, <u>https://doi.org/10.1016/0883-2889(92)90002-V</u>
- W.R. Lacourse, "Pulsed Electrochemical Detection In High Performance Liquid Chromatography", John Wiley & Sons, New York, 1ed, 1997, ISBN 0-471-11914-8
- Chapter 4.1.1. Reagents, European Pharmacopeia, 10.0, (2019), 490
- Chapter 2.2.46, Chromatographic separation techniques, European Pharmacopeia, 11.6, (2024), 6115-6116
- 10. Antec Scientific, Borate ion trap product flyer, <u>https://</u> <u>antecscientific.com/products/columns/borate-ion-trap</u> <u>-column/</u>
- H. Louw, H.J. Brouwer, N. Reinhoud, Electrochemical flowcell, (2016), US patent 9310330 <u>https://patents.google.com/patent/US9310330B2/en</u>
- R.D. Rocklin, A.P. Clarke, M. Weitzhandler, Improved long-term reproducibility for pulsed amperometric detection of carbohydrates via a new quadruplepotential waveform, Anal. Chem, 70 (1998), 1496 – 1501, <u>https://pubs.acs.org/doi/10.1021/ac970906w</u>

 A.H. Dias et al, "Clinical validation of a populationbased input function for 20-min dynamic whole-body ¹⁸F-FDG multiparametric PET imaging", EJNMMI Physics, (2022) 9:60, <u>https://doi.org/10.1186/s40658-022-00490-y</u>

Conclusion

The ALEXYS carbohydrate analyzer in combination with the new SweetSep[™] AEX18 column (USP L46 packing) provides a reliable solution for fast & sensitive analysis of low level FDG impurities in commercial Fludeoxyglucose ¹⁸F Injection samples according to the official EP 11.3 method. The use of the new microbore SweetSep[™] AEX18, resulted in fast separations (< 5 min) with high peak resolution between FDG, FDM and CDG. The presented method has a run time of about 7.4 minutes, which is 3x faster than described in the EP monograph, resulting in a 3-fold increase in sample throughput. 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 of FDG. Moreover, the use of a 2.1 mm ID microbore column minimizes solvent consumption and waste, thus reducing environmental impact.



Ordering information

ALEXYS FDG analyzer (manual injector)		
180.0053WM	ALEXYS FDG Analyzer (incl. SenCell & Clarity CDS software [#])	
ALEXYS analyz	er (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		
260.0051	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	

#) 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.

T) Optional in case a precolumn is preferred, note that the use of a precolumn is not specifically prescribed nor explicitly forbidden in the EP USP monograph [1].

**) In case samples might contain particulate matter it is advised to use a precolumn filter. The pre-column filter PEEK, 0.5 μm (pn 260.0100) includes a starter pack of 4 replacement PEEK frits.

Reagents, standards and sample prep accessories

NaOH 50%, carbonate –free	Fisher Scientific, pn SS254-500
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



Figure 5. 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.

DECADE Elite, ALEXYS, SenCell, FlexCell and HyREF are trademarks of Antec Scientific. Clarity[™] and DataApex[™] are trademarks of DataApex Ltd. Chromeleon[™] is a trademark of Thermo Fisher Scientific. OpenLAB[™] and Chemstation[™] are trademarks of Agilent Technologies, Inc. All other trademarks are the property of their respective owners.

Antec Scientific (USA)

info@AntecScientific.com www.AntecScientific.com T 888 572 0012

Antec Scientific (worldwide)

info@AntecScientific.com www.AntecScientific.com T 31 172 268888

