Application Note

Clinical & Diagnostic

[18F]FDG - Fluorodeoxyglucose - EP USP

- **4 mm ID anion exchange column**
- **Analysis < 16 min**

Introduction

In PET imaging, the radio-labelled tracer 2-deoxy-2-[^{18}F] fluoro-D-glucose, [^{18}F]FDG, can be used for the assessment of glucose metabolism in the human body and tumors in oncology. One of the QC tests that needs to be performed, prior to injection into a patient, is a purity analysis of the synthesized [^{18}F] FDG solution. In such test the actual concentration of the radiotracer and by-products, 2-fluoro-2-deoxy-D-mannose (FDM) and 2-chloro-2-deoxy-D-glucose (CDG) is determined. Compendial methods for this analysis based on High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) are described in both the U.S. Pharmacopeia (USP) and European Pharmacopoeia (EP) [1,2]. In a different application note [3] the impurity analysis of FDG based on the EP and USP method is shown using an Antec ALEXYS Carbohydrate Analyzer in combination with a microbore analytical anion exchange column.

In the present note an example chromatogram for the analysis of FDG and by-products is shown using the traditional standard-bore analytical anion exchange column with larger particles.

Method

The analysis was performed on a ALEXYS Carbohydrate Analyzer (figure 2) consisting of a P6.1L pump with integrated degasser, DECADE Elite electrochemical detector, AS6.1L autosampler and Clarity data acquisition software. The LC conditions from the EP method were used for separation. A SenCell with gold working electrode and HyREF (palladium) reference electrode using a 4-step PAD waveform was used for detection. A summary of the LC-EC conditions is shown in table 1. For detailed information about the method please refer to reference [1-3].

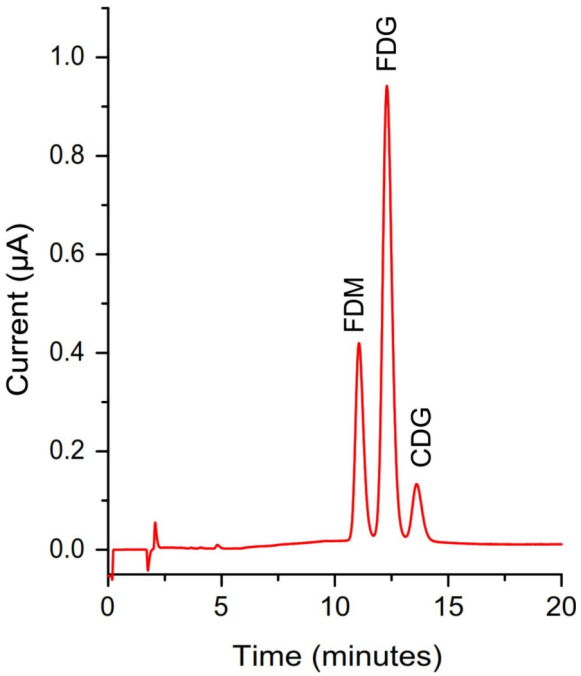

Fig. 1. *Chromatogram obtained with a 10 µL injection of a 50 µg/mL CDG, FDG and FDM standard mixture in water .*

Table 1. LC-EC conditions

HPLC	ALEXYS Carbohydrate Analyzer
Column	CarboPac™ PA10 column, 50 x 4.0 mm ID + 250 x 4.0 mm ID, 10 µm particles All columns: Thermo Scientific™ Dionex™
Mobile phase	100 mM NaOH blanketed with Helium 5.0
Flow rate	1 mL/min
Backpressure	About 150 bar
Injection	10 µL
Temperature	35 °C for separation & detection
Flow cell	SenCell Au HyREF, AST setting 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
I-cell	about 250 nA
ADF	0.1Hz
Range	20 µA/V

Results

An example chromatogram obtained with an 10 µL injection of a 50 µg/mL CDG, FDG and FDM standard mixture in water is shown in figure 2. The relative retention time for FDM, FDG and CDG are 0.9, 1.0 and 1.1 respectively and correspond to the values indicated in the EP monograph. It is evident that the analysis time shown in this note (using a standard bore anion exchange column) is about a factor 2 longer when comparing with the results obtained on a different type of anion exchange column [3]: on that column, all components eluted within 7 minutes (under comparable LC conditions).

For both FDM and FDG, and FDG and CDG, the peak resolution is 1.7 and meets the EP system suitability requirement for (resolution between FDM and FDG ≥ 1.5). The Limit of Detection (LOD) for FDG was about 70 ng/mL. The LOD (3x the ASTM noise) was calculated based on the response of FDG obtained from a 10 µL injection of a 500 ng/mL standard.

Conclusion

The presented data demonstrate that, despite the longer analysis time, the tested column type is a suitable alternative for the analysis of FDG and by-products. In addition, this column is listed in the USP chromatographic database [4] as an L46 phase and is a 'safe' choice when following the compendial method for the CDG impurity analysis (Limit-of-2-chloro-deoxy-d-glucose) described in the EP or USP.

References

1. Fludeoxyglucose F18 Injection, European Pharmacopoeia (EP), 10.0 (2019), 1190-1192
2. Fludeoxyglucose (^{18}F) Injection, The United States Pharmacopeia 43th ed., National Formulary 38th United States Pharmacopeial Convention, Rockville MD, (2019), 1946
3. Antec Scientific, Fluorodeoxyglucose [^{18}F]FDG Radiotracer Analysis, Application note, 217.038
4. USP Chromatographic Database, https://www.usp.org/resources/chromatographic-columns

Ordering information

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
Optional*	
186.ATC00	CT 2.1 column thermostat

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

*) Depending on the lab ambient conditions an optional CT 2.1 column thermostat (186.ATC00) may be required to separate at temperatures between 25 - 30°C.

Fig. 2. *ALEXYS Carbohydrate Analyzer*

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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.