



The soundest LC-EC
Applications for
Clinical & Diagnostics
Analysis ever

Catecholamines

- Serotonin
- Metanephrines
- VMA
- HVA
- 5-HIAA
- Homocysteine
- Glutathione
- (di-)sulfides
- Iodide
- Vitamins A, C, D, E, and K
- Q10
- Ubiquinols

Fluorodeoxyglucose

-
- **Glucose, FDG, FDM and CDG**
 - **PET imaging tracer**
 - **Based on European Pharmacopoeia 6.2, 2008**
 - **Reproducible & Robust**
-

Introduction

In PET imaging, 2-[¹⁸F]fluoro-2-deoxy-D-glucose (FDG) can be used for the assessment of glucose metabolism in the heart, lungs, and the brain. It is also used for imaging tumors in oncology, where usually dynamic images are analyzed in terms of Standardized Uptake Values. The 109.8 minute half-life of ¹⁸F makes rapid and automated chemistry necessary; therefore the FDG is produced in a cyclotron in vicinity of the PET facility.

One of the tests that needs to be performed on the solution before it can be injected into a patient, is to check for the actual concentration of FDG, and the presence of the by-products 2-fluoro-2-deoxy-D-mannose (FDM) and 2-chloro-2-deoxy-D-glucose (CDG).

For the analysis of FDG, the EP [1] requires a 'detector suitable for carbohydrates'. A detector that is sensitive enough and easy to use is pulsed amperometric detector (PAD) [2].



Summary

A method is described for the analysis of FDG using the ALEXYS FDG Analyzer. The European Pharmacopoeia 6.2, (2008) was taken as a basis for the development of this application. The method shows excellent detection limits and linearity. It meets the EP requirements for selectivity as also the metabolites FDM and CDG are measured.

For pulsed electrochemical detection a FLEXCELL has been used which has an exchangeable working electrode, and a maintenance free reference electrode.



Figure 1: ALEXYS FDG Analyzer.

Method

Because the number of samples per day is limited, the ALEXYS FDG Analyzer has been configured with a manual valve. The main elements of this analyzer are an almost pulse free HPLC pump, a polymer based column (resistant to the mobile phase with pH 13), and a DECADE Elite electrochemical detector with a gold working electrode in the flow cell. Helium degassing is advised for reproducible anion exchange chromatography.

Table 1

Conditions	
HPLC	ALEXYS FDG analyzer
Flow rate	1 mL/min
Mobile phase	4 g/L NaOH in water (100 mmol/L)
Column	ALC-525 (250x4.6mm, 7um)
Flow cell	Flexcell with Au WE and HyREF
Temperature	35 °C for separation and detection
Range	50 µA/V
I-cell	0.5 - 1 µA
E1, E2, E3	50 mV, 750 mV, -800 mV
t1, t2, t3, ts	0.5 s, 0.13 s, 0.12 s, 60 ms
Range	50 µA/V

Table 2

EP system suitability requirement		
Parameter	EP criteria	Measured
Resolution FDG-FDM	>1.5	1.75
s/n of FDG	>10	373

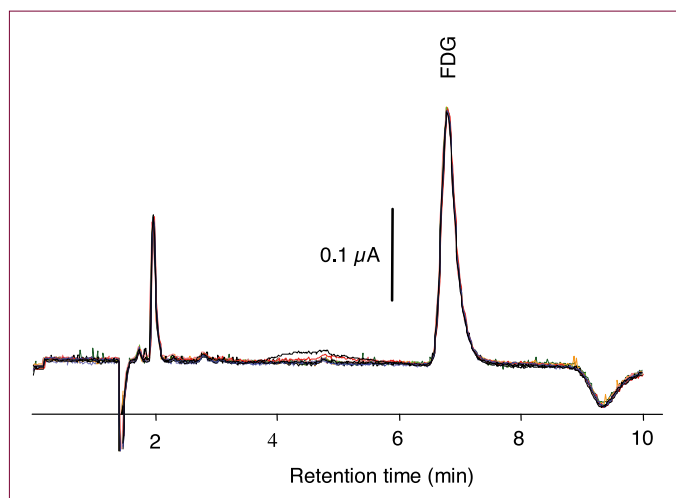


Figure 2: FDG sample (20 µM, 20 µL injected). Overlay of 10 chromatograms. Flow rate: 1.5 mL/min.



Linearity, Repeatability and Detection Limit

Linearity of FDG was investigated in the concentration range of 5 – 1000 μM . The correlation coefficient was better than 0.999 for peak areas and peak heights in this range, as well as sub-ranges.

The relative standard deviation (RSD) in peak area for 10 replicate injections of FDG was 1.4%. The RSD for the retention times was better than 0.2%. The detection limit of FDG is 1 μM .

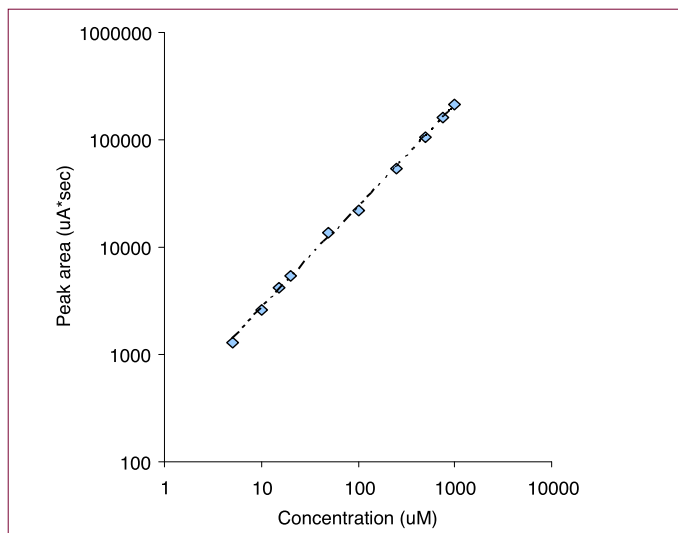


Figure 3: . Response plot of FDG.

EP requirements

In the EP monograph for FDG [1], a system suitability requirement is specified for resolution between FDG and FDM, and signal-to-noise ratio of FDG. The 'reference c solution' was prepared according to the guidelines given in the EP, taking into account a 'maximum recommended dose, in millilitres' of 20 mL. In this case, reference solution c has a final concentration of 250 mg/L FDM and 12.5 mg/L FDG (=1.3 mM FDM and 70 μM FDG).

In Table 2 the EP requirement is compared with the typical results obtained with ALEXYS FDG Analyzer, on the basis of the analysis of 'reference solution c'.

It is evident from Fig. 4 that the detection limit and separation requirement as summarized in Table 2 are easily met by the ALEXYS FDG Analyzer.

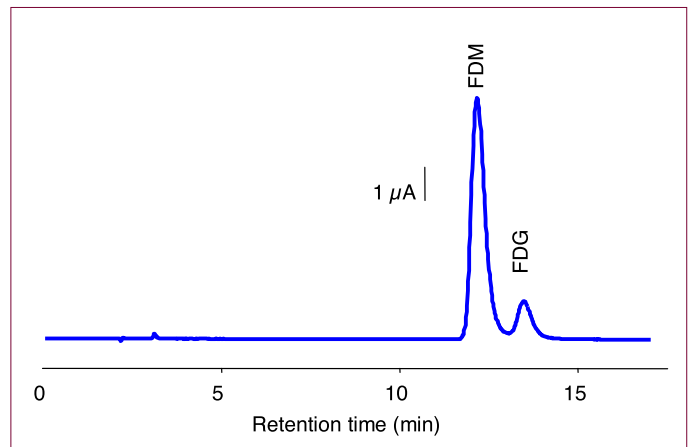


Figure 4: 'Reference solution c', with a final concentration of 1.3 mM FDM and 70 μM FDG, 20 μL injected.

Conclusion

The ALEXYS® FDG analyzer provides a reliable solution for the analysis of FDG and its by-products. It meets the EP requirements for separation and sensitivity.



By-products of FDG production

According to the European Pharmacopoeia [1], the FDG solution needs to be tested among others for impurity 'A', which is CDG. Fig. 5 shows the chromatogram with the signals of Glucose, FDG, FDM and CDG.

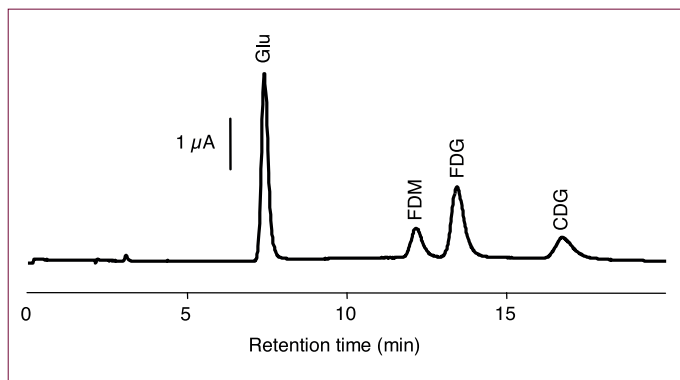


Figure 5: Mix of 50 μM Glucose, 100 μM FDM, 100 μM FDG and 100 μM CDG in water (20 μL injected).

References

1. Fludeoxyglucose (18F) Injection, European Pharmacopoeia 6.2, (2008) 3678-3680
2. W.R. Lacourse, "Pulsed Electrochemical Detection In High Performance Liquid Chromatography", John Wiley & Sons, New York, 1ed,1997.

PART NUMBERS AND CONFIGURATIONS

180.0053E ALEXYS FDG Analyzer with manual injector

Reordering information for application specific consumables

250.1080 ALC-525 Anion Exchange column, 250 x 4.6mm, particle size 7 μm

250.1084 ALC guard column replacement cartridges (5/pk)

250.1700 In-line filter (aqueous)

Distributor:

ERC GmbH

+49 89 66055696

Fax +49 89 60824826

partner@erc-hplc.de

www.erc-hplc.de



For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system. The application was developed with the European Pharmacopoeia 6.2, (2008) as a basis. Column type and actual conditions differ slightly from the EP method. The actual performance may be affected by factors beyond Antec Leyden's control. Specifications mentioned in this application note are subject to change without further notice.

www.myAntec.com

