Determination of FDG and CLDG with Pulsed Electrochemical Detection on a Gold Electrode

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Overview

Purpose: A simple, rapid and accurate method was developed for the analysis of 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG) in radiopharmaceutical products used clinically for positron emission tomography (PET) studies.

Method: Anion exchange with pulsed amperometric detection under basic conditions. Simple dilute and shoot analysis.

Results: The method showed a complete separation of FDG and CLDG, thereby meeting current standard protocols, in less time than current methods and with accuracy and precision below 1.0 µg/mL. Electrode stability over time is clearly demonstrated.

Introduction

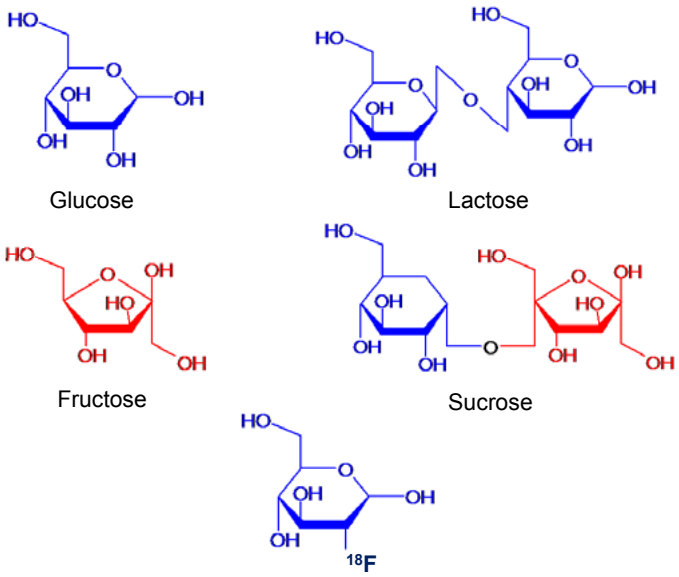
2-Deoxy-2-[^{18}F]fluoro-D-glucose (FDG), shown in Figure 1, is the most widely used radiopharmaceutical for positron emission tomography (PET) studies. This approach is used in the diagnosis of a number of diseases and disorders including cancer, cardiovascular disease, and dementia. In addition, it is an important tool in neuroscience to enable the study of brain regional activation, and in drug research where it is used to evaluate drug biodistribution.

The synthesis of FDG involves nucleophilic displacement and hydrolysis.[1] A contaminant resulting from both processes is 2-deoxy-2-chloro-D-glucose (CLDG). Limits for the presence of ClDG have been set by the U.S. Pharmacopeia (USP), Chemistry, Manufacturing, Controls (CMC), European Pharmacopeia (EP), and soon, the Food and Drug Administration (FDA).[2]

A simple, rapid and accurate method for the measurement ClDG and FDG is described. The method uses high performance anion exchange chromatography (HPAEC) in combination with pulsed amperometric detection (PAD). HPAEC-PAD is a well-established, sensitive, and selective method for determining carbohydrates. The separation is accomplished on a PA20 column using an alkaline mobile phase (50 mM NaOH). Due to the improved sensitivity and selectivity of PAD over other techniques, extensive sample preparation procedures are not required. Samples are simply diluted prior to analysis.

The method allowed complete separation of the FDG and ClDG, meeting the USP resolution criteria of not less than 1.5. The separation was completed in less than 10 minutes and both FDG and ClDG were detected with accuracy and precision at concentrations below 1.0 µg/mL. A problem that has plagued this determination is a loss of analyte response over time due to instability of the electrode response. This poster will clearly demonstrate both chromatographic and signal stability for extended periods of time.

FIGURE 1: Structures of simple carbohydrates and FDG.



Method

Carbohydrate analysis by HPAEC-PAD:

Pump:	Thermo Scientific Dionex ISO-3100 SD
Autosampler	Thermo Scientific Dionex WPS-3000TSL Analytical Autosampler
Flow:	Isocratic at 0.50 mL/min. with constant He purge
Column:	Thermo Scientific Dionex CarboPak: PA20, 3 × 150 mm, 6.5 µm and Hamilton RCX-10 , 4.6 × 250, 7.0 µm
Temperature:	32 °C
Injection volume:	50 µL partial loop
Mobile phase:	50 mM Sodium hydroxide (NaOH), prepared from pellets, 99.99%, semiconductor grade
EC detector:	Thermo Scientific Dionex Coulochem III with Thermo Scientific Dionex Model 5040 cell with Au target: 25 micron Mylar;
EC parameters:	E1: +200 mV, 500 mSec AD: 300 mSec: E2: -2000, 10 mSec E3: +600, 10 mSec E4: -100, 10 mSec
Range:	200 nC
Data analysis:	Thermo Scientific Dionex Chromeleon 6.8 SP11 CDS
Sample preparation	Samples were diluted 1:100 with water prior to injection.

Results and Discussion

Carbohydrates are commonly measured using high performance anion exchange chromatography in combination with pulsed amperometric detection (PAD). However, in order for the analytical system to perform optimally, a number of important points must be taken into consideration:

• The HPLC system must be compatible with basic mobile phases. Titanium components cannot be used as they will degrade leading to column contamination (changes in retention times) and working electrode fouling (loss of response). Stainless steel-based systems work well once thoroughly passivated.

• Exposure of the mobile phase to carbon dioxide must be kept to a minimum. The formation of carbonate leads to both chromatographic and detection issues. Mobile phase should be made fresh and must be actively degassed or sparged with helium. Mobile phase bottles must be made from Teflon® (other plastics such as PE are gas pervious). All liquid lines must be gas impervious.

• Do not use glass as it dissolves when exposed to base and will lead to issues with chromatographic separation and detection. Mobile phase should be stored in PTFE bottles and not filtered through a glass-based filtration device.

• Reagents and water must be pure. Sodium hydroxide (NaOH), pellets, 99.99%, semiconductor grade (p/n 306576, Sigma-Aldrich, Milwaukee WI) is highly recommended. Water at 18.2 MΩ·cm must be used.

• Do not use solvents such as methanol or isopropanol as these will lead to high back ground currents and will adversely affect the method. The autosampler wash solution should use water with a microbicide (Reagent MB) in order to prevent microbial growth and system contamination.

During the first phase of the study, the compatibility of the stainless steel pump and autosampler with high base was studied. A HPAEC-PAD method for the measurement of simple carbohydrates (glucose, fructose, lactose and sucrose) was used. The non-titanium system performed excellently. The chromatogram shown in Figure 2 illustrates replicate injections of carbohydrate standards (1 ng on-column). The sensitivity of this method reached a limit of detection (LOD) of less than 100 pg on-column (data not shown). To determine retention time and response stability, a 50 ng injection was made every hour for 14 hours. Good retention stability and response reproducibility were observed (Table 1). Response curves for simple sugars with concentrations ranging from 2–200 µg/mL are shown in Figure 3. The correlation coefficients for these calibrations were 0.99 or higher.

FIGURE 2: Analysis of carbohydrates using HPAEC-PAD. Triplicate injections of 1 ng simple carbohydrates.

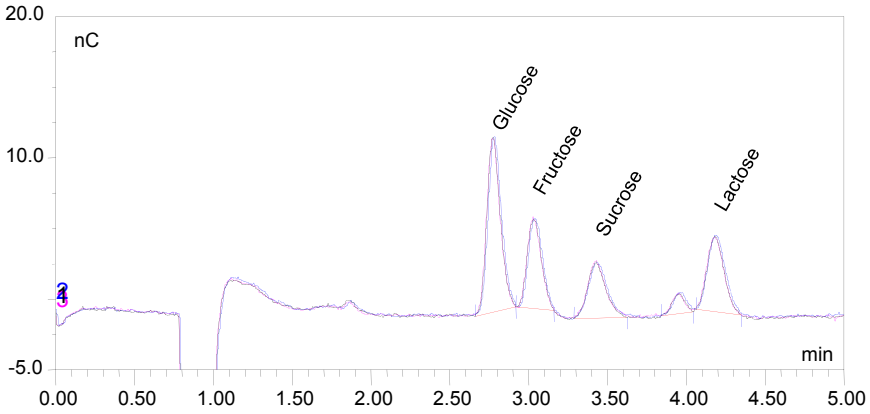
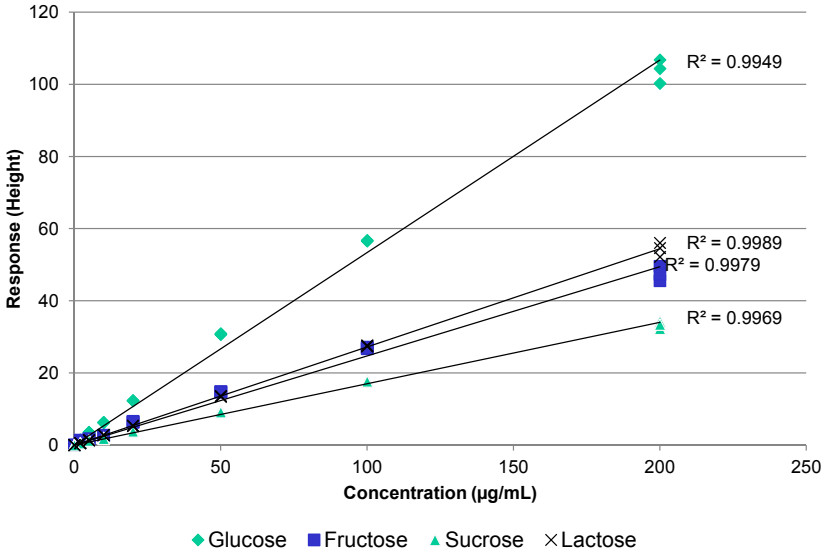


Table 1: Reproducibility of detector response over 14 hours for the analysis of simple carbohydrates (50 ng injected).

	Glucose		Fructose		Sucrose		Lactose	
	Ht	Area	Ht	Area	Ht	Area	Ht	Area
Mean	178.3	15.9	85.9	8.7	44.6	5.1	95.2	12.9
SD	1.61	0.21	0.93	0.19	0.51	0.11	0.90	0.21
RSD	0.90	1.33	1.08	2.15	1.15	2.23	0.95	1.59

FIGURE 3: Response curves for simple carbohydrates.



Once the stability of the analytical system was confirmed, the second part of the study was to develop a method capable of simultaneously measuring FDG and low levels of the impurity CIDG with the limits set by agencies previously mentioned. The HPAEC-PAD method using the CarboPak™ anion exchange column was completed within 8.5 mins (Figure 4). The quantitative limit (LOQ) for the determination of FDG and CLDG was about 50 ng on-column. Due to the sensitivity and selectivity of the PAD method, extensive sample preparation procedures were not required, the samples simply being diluted prior to analysis. The reproducibility of the chromatographic separation and detector response were evaluated by injecting the 10 µg/mL standard every two hours for 24 hours. The results are shown in Table 2.

FIGURE 4: Standard illustrating the separation and detection of 10 µg/mL FDG, CLDG, and mannose using the CarboPak PA-20 column.

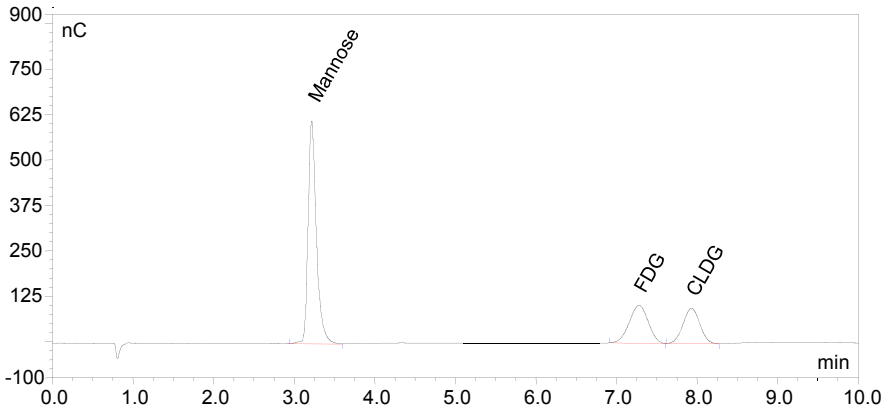


Table 2: Reproducibility of detector response over 24 hours for the analysis of FDG, CIDG, and mannose using the CarboPak PA-20 column.

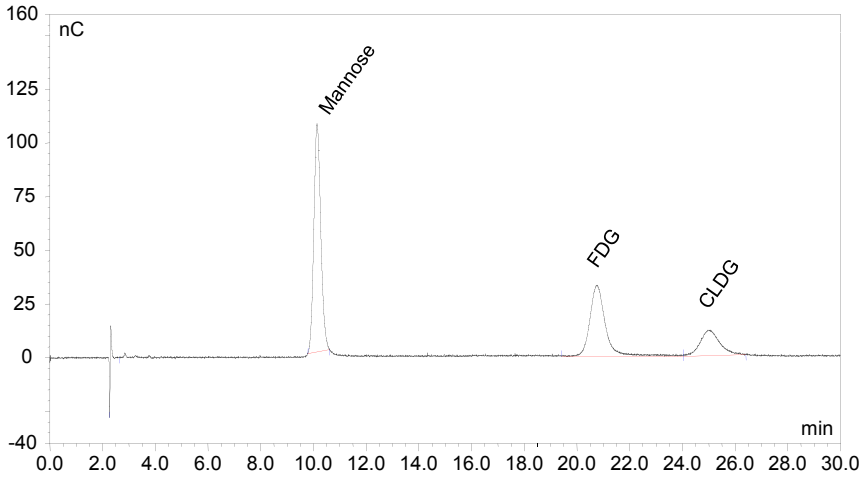
Time	Mannose			FDG			CLDG		
	RT	HT	AREA	RT	HT	AREA	RT	HT	AREA
Mean	4.05	652.38	103.85	9.16	523.62	181.85	9.94	439.08	139.77
SD	0.03	18.03	1.99	0.10	17.61	4.14	0.11	8.05	1.69
RSD	0.79	2.76	1.92	1.13	3.36	2.28	1.08	1.83	1.21

A second column, a Hamilton RCX-10 was also evaluated for comparison purposes. Initially, issues with retention time stability were observed with the RXC-10 anion exchange column. This was eventually traced to a titanium frit in the guard column, precluding the pre-column from further use. The Hamilton RCX-10 column provided a slightly different degree of selectivity, with more resolution between FDG and CLDG than the CarboPak, but required a much longer run time (Figure 5). The overall reproducibility of over 8 hours for the Hamilton column over an eight-hour period is presented in Table 3. Although acceptable, there was slightly more variation in these parameters than with the CarboPak column.

Table 3: Reproducibility of detector response over 8 hours for the analysis of FDG, CIDG, and mannose using the Hamilton RCX-10 column.

Time	Mannose			FDG			CLDG		
	RT	HT	AREA	RT	HT	AREA	RT	HT	AREA
T = 0 Hr	10.3	107	33.2	21.3	32.5	21.4	25.7	11.1	10.5
T = 8 Hr	9.91	103	31.0	20.1	31.7	19.3	24.2	11.9	9.7

FIGURE 5: Standard illustrating the separation and detection of 10 µg/mL FDG, CLDG, and mannose using the Hamilton RCX-10 column.



Conclusion

- Using high performance anion exchange chromatography with the Coulochem™ III electrochemical detector and CarboPac columns provided a routine and robust approach for the direct determination of simple carbohydrates. With a relative standard deviation (RSD) of less than 2% (height) over a fourteen hour period, the data clearly demonstrates that method is free from the decreased response issues seen with other approaches. The mass sensitivity of this method allowed for detection of 100 pg simple carbohydrates.
- A rapid method for the simultaneous analysis of the most widely used radiopharmaceutical FDG and its impurity ClDG was developed.
- The original USP monograph described the use of a liquid chromatograph system equipped with a pulsed amperometric detector and a 4.0 mm × 25 cm column that contains 10 µm packing L46.
- The USP monograph indicates that the resolution, *R,* between FDG and ClDG must not less than 1.5. The method meets this criteria.
- The RSD for replicate injections must not be more than 5%. A RSD of less than 2% over a fourteen hour period exceeded specifications.
- The original FDG method that used a Hamilton column and evaluated in our study did achieve these criteria and did not show response issues once the problem with the guard column was addressed. However, the analysis time was long, taking more than 26 mins to complete.
- The CarboPak PA-20 anion exchange column performed very well and met all the criteria of the USP method, and had the advantage that the analysis time was short, just 8.5 mins to complete.

References

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2. Fluorodeoxyglucose F 18 Injection. *The United States Pharmacopeia, 29th ed. and The National Formulary, 24th* . Rockville (MD): United States Pharmacopeial Convention, Inc,; 2006. 933-4.

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