INFLUENCE OF THE SEPARATION PARAMETERS APPLIED FOR DETERMINATION OF IMPURITIES FDG AND CLDG

MIRELA MIHON1,2, CATALIN STELIAN TUTA2, ALINA CATRINEL ION3, JACEK KOZIOROWSKI3, DANA NICULAE2, VASILE LAVRICE1, DOINA DRĂGĂNESCU2,4*

1“Politehnica” University of Bucharest, Faculty of Applied Chemistry and Materials Science, 1-7 Polizu Street, RO-011061, Bucharest, Romania
2“Horia Hulubei” National Institute for Physics and Nuclear Engineering, 30 Reactorului Street, Măgurele, Ilfov, Romania
3Department of Radiation Physics and Department of Medical and Health Sciences, Linköping University, Linköping, Sweden
4“Carol Davila” University of Medicine and Pharmacy, Faculty of Pharmacy, 6 Traian Vuia Street, RO-020956, Bucharest, Romania

*corresponding author: doinadraganescu@yahoo.com

Manuscript received: January 2016

Abstract
2-fluoro-2-deoxy-D-glucose (FDG) and 2-chloro-2-deoxy-D-glucose (CIDG) are chemical impurities found in the 2-[18F]fluoro-2-deoxy-D-glucose products (18F-FDG). The objective of this study was to find the best condition for the separation of FDG and CIDG, evaluating different columns under various operating conditions. Chromatographic parameters such as column temperature, composition and flow rate of the mobile phase were the independent variables used in the optimization process. The optimized method was validated and validation results showed a good accuracy, repeatability and reproducibility.

Rezumat
2-fluoro-2-deoxy-D-glucoza (FDG) și 2-chloro-2-deoxy-D-glucoza (CIDG) sunt impurități chimice care se găsesc în produsele farmaceutice marcate radioactiv ce conțin 2-[18F]fluoro-2-deoxy-D-glucoza (18F-FDG). Obiectivul acestui studiu a fost de a găsi cea mai bună metodă de separare a FDG și CIDG prin evaluarea diferitelor coloane în condiții de operare variate. Parametrii cromatografici cum ar fi temperatura coloanei, compoziția și debitul fazei mobile au fost variabile independente utilizate în optimizare. Metoda astfel optimizată a fost validată, iar rezultatele au demonstrat o bună precizie, repetabilitate și reproducibilitate.

Keywords: Chemical impurities, radio-HPAEC, validation

Introduction
Chemical impurities are considered all nonradioactive substances that can affect radiolabelling. Since synthetic methods are used in the preparation of Positron Emission Tomography (PET) compounds, analysis of chemical purity is necessary due to the adverse reactions which might be generated within the system [3, 12].

Chemical impurities are species which have similar biological activity and compete with the radiotracer for binding to the specific receptor or antigen, thus reducing the in vivo apparent or effective specific activity (SA). Chemical contaminants resulted from side reactions are species which may be toxic for the organism subjected to a radiolabelling. Such a potential impurity is 2-chloro-2-deoxy-D-glucose (CIDG) which should be less than 0.05 mg/mL in the 2-[18F]fluoro-2-deoxy-D-glucose products (18F-FDG) product. The former might appear when an anion exchange resin in chloride form (quaternary ammonium anion cartridge) is used during synthesis and, possibly, from acid hydrolysis. Therefore, the FDG solution needs to be tested for both contaminants and impurities. 18F-fluoro-deoxy-mannose is rarely found in notable amounts as 18F-FDG is stable towards epimerization under normal reaction condition [7-9].

The aim of this research was to select the best stationary phase for FDG and CIDG determination amongst different columns and to optimize the chromatographic parameters (concentration and flow rate of the mobile phase and column temperature).

The first separation of FDG and CIDG by ion exchange was described by Alexoff [1]. Previously reported protocols for anion exchange chromatography often require long time to perform the analysis. The present study presents an efficient stable and reproducible waveform of potential - time parameters.
when using gold electrode as working and Ag/AgCl as reference electrodes.

Materials and Methods

Chromatographic studies
High performance anion-exchange chromatography (HPAEC) measurements were performed on Agilent Bio-Inert 1260 Series equipped with UV-VIS and NaI (TI) scintillation detectors. The HPLC has been configured with a manual injector. The main components of the system consist of a polymer based column (resistant to the mobile phase with pH = 13) and a DECADE II electrochemical detector with a gold electrode in the flow cell. Electrochemical detection was performed in pulsed mode using an Au cell with Ag/AgCl reference electrode. Radioactive samples were analysed using a gamma radioactive detector with NaI cell. The data collection and integration of the chromatographic tests are done in OpenLabChemStation software.

The proposed method consists of two chromatographic column systems: CarboPac PA1 (4 x 250 mm, 10 µm diameter, substrate-poly(styrene 2% crosslinked with divinylbenzene agglomerated with 500 nm Micro-Bead latex) and guard PA1; and CarboPac PA10 (4 x 250 mm, 10 µm diameter, substrate-ethylvinylbenzene 55% cross-linked with divinylbenzene agglomerated with 460 nm MicroBead latex).

Both CarboPac columns are packed with a polymeric nonporous, MicroBead film resin, since the latex exhibits rapid mass transfer, high pH stability in the range 0 - 14 and excellent mechanical strength that permits pressures higher than 4000 psi.

Materials
Solvants and chemicals (p.a grade) were obtained from Merck and ABX.
5 mg of FDG standard were weighted, then transferred accurately into 10 mL volumetric flask and dissolved in water to obtain the standard stock solution. Similarly, 4 mg of CIDG standard were weighted, then transferred accurately into 10 mL volumetric flask and dissolved in water to obtain the standard stock solution.

Working standard solutions were obtained by serial dilution of standard stock solutions.

Validation study
The validation of the analytical methods was carried out according to ICH guidelines. The parameters used in the validation process were: system suitability, specificity, precision, accuracy and linearity, limit of detection (LOD) and limit of quantification (LOQ) [4, 11].

The system suitability was evaluated by injecting blank solution (duplicate) and standard test solution (six injections) using the optimized method. The chromatograms were recorded, evaluated and the relative standard deviations were calculated. The resolution between two successive peaks was measured.

The precision was evaluated by injecting six standard test solutions, measuring the response for each peak and calculating its relative standard deviation. When it comes to precision, there are two time-scales of reference: the intra-day and the inter-day. The intra-day precision was evaluated performing the analysis of several replicates of the reference standard solution having a concentration of 50 µg/mL by the same operator during a single day, while inter-day precision was assessed using different operators who applied the methodology in different days.

The method’s accuracy was validated through recovery experiments by spiking with known amount of each impurity at 80%, 100% and 120%. Each concentration was prepared in triplicate and the percent recovery was calculated.

LoD and LoQ were calculated for these impurities, based on the Standard Deviation of Response (σ) and Slope (S) methodology.

System suitability was evaluated by injecting the blank solution and standard test solution (six injections) using the optimized method. The chromatograms were recorded, evaluated and the relative standard deviation was calculated. Common system suitability parameters include: asymmetry, resolution and efficiency.

Results and Discussion

HPAEC methods were developed based on the chromatographic separation of standards FDG and CIDG considering the shorter retention time (faster analysis) and better separation (resolution Rs > 1). The European Pharmacopoeia (Eur.Ph) specifies that the chemical purity of FDG and CIDG should be determined by HPLC using a strong basic anion exchange resin. The mobile phase is 0.1M NaOH and the flow rate is 1 mL/min. In analysis of FDG the Eur.Ph requires “a detector suitable for carbohydrates”.

Thus, a corresponding system that is sensitive enough and easy to use is the pulsed amperometric method (PAD) column [5, 6]. To determine the optimum detector response, the detection potential was changed systematically from 0.1 V to 0.2 V for all columns. A number of parameters were varied in the HPAEC system: temperature of the column and the mobile phase’s flow rate and concentration.

Applying the above mentioned procedure, the parameters of the optimized method were found, as presented in Table I, while the details are presented further.
Table I
Chromatographic parameters for the optimized HPAEC/PAD method

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow (mL/min)</td>
<td>1</td>
<td>Isocratic 0.5 - 1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Column</td>
<td>strongly basic anion-exchange resin</td>
<td>PA1, PA1 and guard column PA1; PA10</td>
<td>PA10</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>20 - 30</td>
<td>25 - 55</td>
<td>40</td>
</tr>
<tr>
<td>Injection volume, µL</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>0.1M Sodium hydroxide (NaOH)</td>
<td>0.1 M - 0.2 M</td>
<td>0.1 M</td>
</tr>
<tr>
<td>Detector</td>
<td>detector suitable for carbohydrates</td>
<td>PAD Working potential 0.1 V - 0.2 V vs. Ag/AgCl reference electrode</td>
<td>0.15</td>
</tr>
<tr>
<td>Range, µA</td>
<td>-</td>
<td>50 - 200</td>
<td>200</td>
</tr>
</tbody>
</table>

Optimization of the sodium hydroxide concentration
Variation of the sodium hydroxide concentration did not influence the separation. The sensitivity of the detection decreased with increasing its concentration. Consequently, the concentration of 0.1 M NaOH was considered appropriate.

Effect of the column temperature
Separation on CarboPac PA1 was performed at flow rates in the range 0.5 - 1.1 mL/min, detection potentials between 0.1 V and 0.2 V, and a low temperature (25°C) and high temperature (55°C). CarboPac PA10 was operated at the same conditions. The results obtained with CarboPac PA1 for temperature 40°C and 55°C are shown in Figure 1.

![Chromatograms for FDG and ClDG standards – column PA1 (a) 40°C (b) 55°C](image1.png)

Figure 1.

We performed the analysis at different temperatures, while other conditions were kept constant. The heating temperature on the analytical column would improve the separation efficiency, due to the better mass transfer of analytes among the packing latex of analytical columns. To evaluate the effect of the column temperature on the sensitivity of the FDG and ClDG measurements, a standard mixture of 50 µg/mL FDG and 50 µg/mL ClDG was tested varying column temperatures (25, 30, 35, 40, 45, 55°C). In accordance to Nakao et al. [10] at temperatures over 40°C the peak area of ClDG decreases gradually, but with increasing the column temperature the FDG peak became sharper and the resolution was better (Figure 2).

![Chromatograms for FDG and ClDG standards at different temperatures for PA10 column (a) 40°C (b) 55°C](image2.png)

Figure 2.
Effect of mobile phase flow rate on the detection of impurities

The separation of FGD and CIDG was performed at flow rates in the range 0.5 mL - 1.1 mL/min using both columns Dionex CarboPac PA1 and Dionex CarboPac PA10 at 40°C (Table II).

### Table II

<table>
<thead>
<tr>
<th>Mobile phase flow rate</th>
<th>CarboPac PA1 Retention Time</th>
<th>CarboPac PA10 Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FDG</td>
<td>CIDG</td>
</tr>
<tr>
<td>0.5 mL/min</td>
<td>16.81</td>
<td>17.79</td>
</tr>
<tr>
<td>0.8 mL/min</td>
<td>10.81</td>
<td>11.36</td>
</tr>
<tr>
<td>1 mL/min</td>
<td>8.25</td>
<td>8.81</td>
</tr>
<tr>
<td>1.1 mL/min</td>
<td>7.82</td>
<td>8.01</td>
</tr>
</tbody>
</table>

For CarboPac PA1 column the best resolution was obtained using 0.5 mL/min flow rate; the inconvenience it is the time of analysis which increase until 30 minutes (Figure 3).

![Figure 3](image)

**Chromatograms for FDG and CIDG standards at different flow rates for PA1 column**

a) flow rate 0.8 mL/min b) flow rate 0.5 mL/min

At the flow rate 0.5 mL/min, the resolution was improved but the retention time for CIDG was 15.01 min, with a total run of analysis of 30 minutes (Figure 4).

![Figure 4](image)

**Chromatogram for FDG and CIDG standard in CarboPac PA10, temperature of 40°C**

a) flow rate of 0.5 mL/min and b) flow rate 0.8 mL/min

The best results were achieved using CarboPac PA10 column at a temperature of 40°C and a flow rate 0.8 mL/min. The sodium hydroxide concentration of the mobile phase was kept constant at 0.1 M. The total run was 15 min (Figure 4b).

**Method validation for FDG and CIDG impurities**

For further validation of the method the column CarboPac PA10, the flow rate 0.8 mL/min and the column temperature 40°C were selected.

The optimization of three potential-time wave forms was achieved (Figure 5a) and the validation of this analytical procedure showed its adequacy for determination of the chemical purity of $^{18}$F-FDG (Figure 5b).
Figure 5.

a) The optimal three potential-time waveforms for HPAEC/PAD
b) Calibration curves for FDG and ClDG standards

<table>
<thead>
<tr>
<th>Table III Validation parameters of the optimized method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Validation Parameter</strong></td>
</tr>
<tr>
<td><strong>Linearity:</strong> The correlation coefficient ($R^2$) should be $&gt; 0.99**</td>
</tr>
<tr>
<td><strong>FDG</strong></td>
</tr>
<tr>
<td>Regression Equation</td>
</tr>
<tr>
<td>Correlation coefficient ($R^2$)</td>
</tr>
<tr>
<td>Range ($\mu$g/mL)</td>
</tr>
<tr>
<td><strong>Precision:</strong></td>
</tr>
<tr>
<td><strong>Repeatability</strong></td>
</tr>
<tr>
<td><strong>Intra-day precision</strong></td>
</tr>
<tr>
<td>80%</td>
</tr>
<tr>
<td>100%</td>
</tr>
<tr>
<td>120%</td>
</tr>
<tr>
<td><strong>LoD and LoQ values</strong></td>
</tr>
<tr>
<td>LoD ($\mu$g/mL)</td>
</tr>
<tr>
<td>LoQ ($\mu$g/mL)</td>
</tr>
<tr>
<td><strong>Accuracy:</strong> % Recovery should be within 90 - 110%</td>
</tr>
<tr>
<td>Concentration (%)</td>
</tr>
<tr>
<td>80</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>120</td>
</tr>
</tbody>
</table>

**Precision, linearity and suitability evaluation of the optimized method**

The last step was to evaluate the precision, linearity and suitability of the optimized method, according to the further details.

When it comes to precision, there are two time-scales of reference: the intra-day and the inter-day. The intra-day precision was evaluated performing the analysis of several replicates of the reference standard solution having a concentration of 50 $\mu$g/mL by the same operator during a single day, while inter-day precision was assessed using different operators who applied the methodology in different days. The precision of the proposed method was good (RSD $< 5\%$) in conformity with ICH guideline. The RSD in peak areas for 6 replicate injections of FDG and CIDG was 1.84 and 3.33.

Linearity of the FDG and CIDG was investigated in the concentration range of 4 - 100 $\mu$g/mL (FDG) and 6 - 100 $\mu$g/mL (CIDG). According to the calibration curves, the correlation coefficients were greater than 0.99 for peak areas and peak heights in this range (Figure 5b).

The specificity of the method was performed by injecting standard solution of CIDG and FDG and a mixture of standard solution for these compounds and comparing the retention time of each standard (resolution Rs > 1).
System suitability was evaluated by injecting the blank solution and standard test solution (six injections) using the optimized method. The chromatograms were recorded, evaluated and the relative standard deviation was calculated. Common system suitability parameters include: asymmetry, resolution and efficiency.

The recovery of FDG and CIDG in 3 diluted samples with concentration of 80%, 100% and 120% from the target values of 50µg/mL was determined.

The results obtained for the optimized method are summarized in Table III; the parameters of validation process were evaluated in conformity with ICH and Eur.Ph [2, 4]. The acceptance criteria were generally tighter for the desired compound (98%-102%) than for impurities (95% - 105%). FDG and CIDG are chemical impurities in 18F-FDG. The absolute value for 18F-FDG radioactivity is 90% - 110% (Eur.Ph Monograph).

Conclusions

An useful and fast HPAEC/PAD method for the quality control of 18F-FDG has been developed and validated. The chemical impurities could be separated by anion exchange chromatography with alkaline mobile phase and detected by pulsed amperometric detection. The determination of impurities in short time is an essential step in characterizing of each batch, due to rapid decay of 18F (110 min half-life). The analysis time is short, just 15 min to be completed.

Acknowledgement

“Mirela Mihon acknowledges the support by the Sectorial Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and the Romanian Government under the contract number POSDRU/159/1.5/S/137390”

References


