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EVALUATION OF STEAM STERILIZATION CONDITIONS FOR [^{18}F]FLUDEOXYGLUCOSE

Priscilla. F. Santos, Leonardo T. Nascimento, Eduardo S. Valente, Juliana B. Silva, Marina B. Silveira, Soraya Z. Ferreira

Unidade de Pesquisa e Produção de Radiofármacos
Centro de Desenvolvimento da Tecnologia Nuclear (CDTN / CNEN – MG)
Av. Presidente Antônio Carlos, 6.627, 31270-901
Belo Horizonte, MG
somafe@cdtn.br

ABSTRACT

[^{18}F]Fludeoxyglucose (^{18}FDG) is the most commonly used radiopharmaceutical for positron emission tomography. Sterile filtration of the final product into sterile vials using 0.22 µm filter membrane is usually adopted for ^{18}FDG. However, Good Manufacturing Practice (GMP) guidelines recommend heat sterilization as the method of choice to ensure sterility of pharmaceutical preparations. The aim of this study was to essay different steam sterilization conditions in order to choose the best one for ^{18}FDG. Three different sterilization conditions were essayed. The first one at 121 °C for 15 minutes, the second one at 135 °C for 3.5 minutes and the third one at 133 °C for 2 minutes. ^{18}FDG pH-formulation was kept around 6.0. At the end of autoclave cycles, ^{18}FDG sterility was evaluated by direct inoculation of ^{18}FDG in culture media and radiochemical purity was determined by TLC and HPLC. Results demonstrated that all essayed conditions were able to ensure ^{18}FDG sterility, but caused a decrease in radiochemical purity of ^{18}FDG. Autoclave cycle at 133°C for 2 minutes was the best essayed condition for ^{18}FDG terminal sterilization, once it provided the greater radiochemical purity value and took less time. ^{18}FDG was able to meet specifications after autoclave cycles, what supports the application of steam sterilization in routine ^{18}FDG production, in compliance with GMP.

1. INTRODUCTION

^{18}FDG is the most widely used PET tracer worldwide. It is a glucose analogue in which the hydroxyl group on the 2-carbon of a glucose molecule is replaced by a [^{18}F] atom. Clinical applications of ^{18}FDG include therapy monitoring, tumor staging, myocardial energy turnover and many different neurological diseases [1].

In the current practice of PET-tracer production, sterile filtration of the products into sterile vials is the most often used method of sterilization [2]. However, there is a general agreement that heat sterilization is the method of choice to ensure sterility of pharmaceuticals when it is possible [3-5].

The use of steam sterilization is particularly interesting in ^{18}FDG production, once heat is considered the most simple and safe sterilization agent [6] and batches must be released before the completion of sterility test due to short half-life of ^{18}F (109.7 minutes) [7].

Although carbohydrates can be considered heat-labile compounds, it has been reported that steam sterilization of ^{18}FDG is possible if appropriate conditions are settled [2]. In this context, the aim of this study was to essay different steam sterilization conditions in order to choose the best one for ^{18}FDG terminal sterilization.

2. EXPERIMENTAL

2.1. Materials

The reference standard of FDG was obtained from the manufacturer ABX. ^{18}O-enriched water ($H_2{}^{18}O$) was acquired from Center of Molecular Research. Reagent kits containing eluent solution, acetonitrile, ethanol, NaOH 2.0 mol·L^{-1}, buffer solution, water for injections and mannose triflate for ^{18}FDG synthesis were purchased from ABX. NaOH used in HPLC mobile phase was purchased from Sigma-Aldrich. The Carbopac PA column was acquired from Dionex and 0.22 µm filter membranes were purchased from Millipore.

2.2. Methods

2.2.1. ^{18}FDG production

^{18}FDG was synthesized in our laboratory using an automated synthesizer (TRACERlab^{TM} MX_{FDG}, GE) as reported previously [8]. The final product was fractionated and dispensed in glass vials using an automated system (Theodorico, Comecer).

2.2.2. Steam sterilization

The vials containing ^{18}FDG were submitted to thermal sterilization using saturated steam under pressure by means of autoclave (RPA/1, Comecer). Three steam sterilization conditions were proposed and two batches of ^{18}FDG were essayed for all conditions. The first one was set at 121 ºC for 15 minutes in accordance to the Brazilian Pharmacopeia, the United States Pharmacopeia (USP) and the European Pharmacopoeia (EP) [6,7,9]. The second condition was 135 °C for 3.5 minutes, as reported previously [2]. The third one, 133 °C for 2 minutes, was established based on F_0-value calculation according to Equations 1 and 2 [9].

$$F_0 = D_{121}(\log A - \log B) \quad (1)$$

where D_{121} is the time required at 121°C to reduce the population of most heat resistant organism (*Geobacillus stearothermophilus*, ATCC 7953) by 90%; A is the microbial count per container in the beginning of the process and B is the maximum acceptable survival that is $1/10^6$ of the initial microbial count. D-value of the commercial biological indicator Sterikon® (Merck) used was 2 minutes, according to certificate of analysis.

$$F_0 = \sum_{t_1}^{t_2} 10^{\frac{T_0 - T_t}{z}} \Delta t \quad (2)$$

where t_1 is the time at which the temperature passes 121 °C in the heating phase; t_2 is the time at which the temperature falls below 121 °C in the cooling phase; T_0 is the temperature to which the F_0 value is related (121 °C); T(t) is the time-dependent temperature in the sterilization unit; z is the temperature difference in °C that causes an increase of the D value by a factor of 10 and Δt is the time interval of the periodically temperature measurements.

A minimum F_0 value of 30 minutes was established as an internal specification for quality control release. This conservative approach was based on limited temperature measurement precision.

The temperature and pressure in autoclave was monitored throughout the whole sterilization cycle. Heat distribution studies were performed using calibrated thermocouples (K type, Iope). Steam sterilization physicochemical and biological indicators were used to supplement the information obtained through physical assessment of the critical operating parameters. Both indicators were placed in the autoclave along with the batch of ^{18}FDG to be sterilized. After autoclave cycles, ampoules containing a highly resistant spore (*Geobacillus stearothermophilus*, ATCC 7953) and culture media were incubated at 60 °C for 48 hours.

2.2.3. Quality control of steam sterilized ^{18}FDG

Quality control included the evaluation of pH, radiochemical purity and sterility, which were considered the most critical parameters considering the scope of this study. These essays were performed, in triplicate, before and after autoclave cycles, based on methodology described in [7,9].

^{18}FDG pH was determined using a pHmeter S20 (Mettler Toledo). Radiochemical purity was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). TLC chromatogram was developed in a solvent system consisting of acetonitrile and water (95:5) and then scanned using a radiochromatographic TLC scanner (Raytest). HPLC analysis was performed with an HPLC (Agilent). ^{18}FDG and impurities were separated on an anion-exchange column by isocratic elution with NaOH 0.1 $mol \cdot L^{-1}$ as the mobile phase. The flow rate of the mobile phase was set at 0.8 mL / min and the column temperature was kept at 35 °C. Radiochemical purity was determined as the percent fraction of the total radioactivity which corresponds to ^{18}FDG in TLC and HPLC. The test for sterility was carried out under aseptic conditions. Sterility test was performed by direct inoculation of ^{18}FDG sample without dilution into soybean–casein digest medium and fluid thioglycollate medium, followed by incubation for 14 days, at 20-25 °C and 30-35 °C, respectively.

Acceptance criteria for these tests are presented in Table 1. They were established based on Fludeoxyglucose ^{18}F monograph [7], except pH. Although an acceptable range for pH is 4.5-7.5, it was specified as 6.0-6.5 in this study to avoid that a factor other than steam sterilization condition could interfere in radiochemical purity results.

Table 1. Acceptance criteria for pH, radiochemical purity and sterility of ^{18}FDG

Test	Specification
pH	6.0 - 6.5
Radichemical purity	> 90%
Sterility	Sterile

3. RESULTS AND DISCUSSION

The whole sterilization process took about 24, 16 and 14 minutes, respectively, for the first, second and third essayed conditions. As a general procedure, a 3-6-minute heating phase and a 6-minute cooling phase were performed for all conditions. Autoclave cycles were represented in Fig. 1.

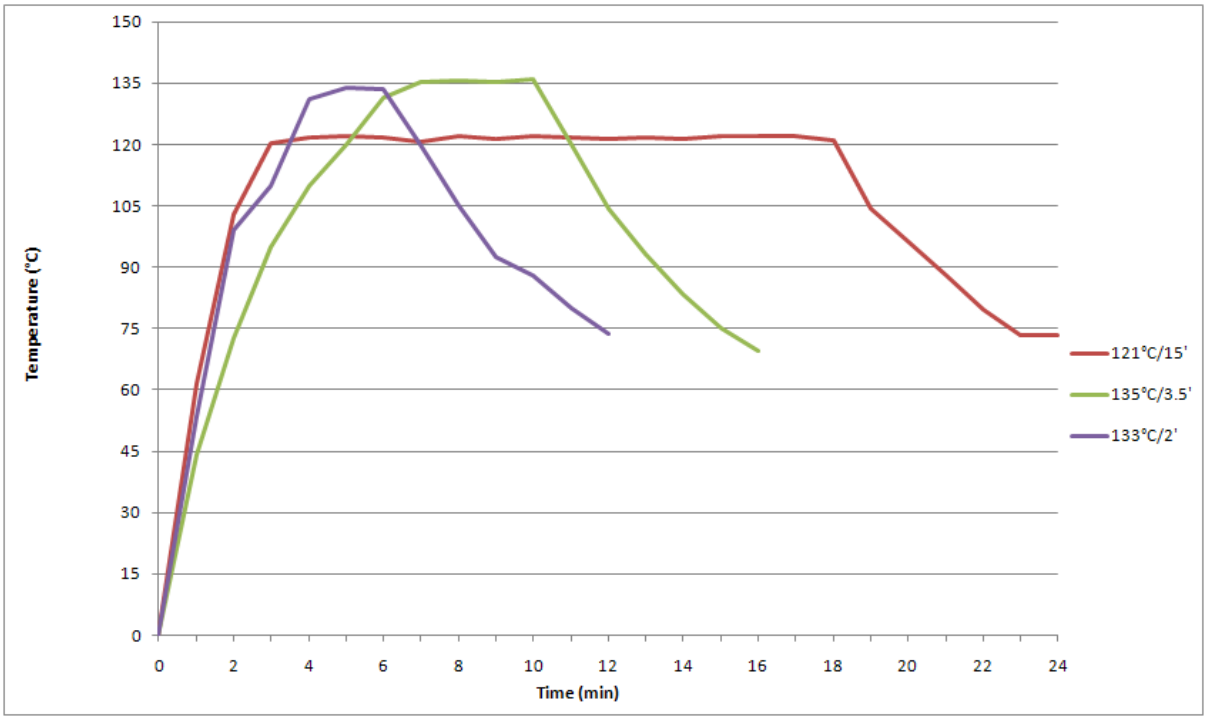

Figure 1. Autoclave cycles with different steam sterilization conditions

The comparative readings of autoclave sensors and calibrated thermocouples differed by less than 1°C during the sterilization phase, which is in accordance to pharmacopeia requirements [6, 7]. Physicochemical and biological indicators confirmed the effectiveness of steam sterilization cycles. Physicochemical indicator changed from light pink to brown as a result of exposition to steam. No evidence of microbiological growth was observed in biological indicators containing *Geobacillus stearothermophilus*.

Results of pH, radiochemical purity and sterility tests performed with two batches of ^{18}FDG before and after autoclave cycles are reported in Table 2 as mean ± standard deviation.

Table 2. Results of ^{18}FDG analysis

Test	Before steam sterilization	121°C / 15 min	135°C / 3.5 min	133°C / 2 min
pH	6.18 ± 0.04%	6.18 ± 0.04%	6.18 ± 0.04%	6.18 ± 0.04%
Radiochemical purity (TLC)	96.7 ± 1.1%	96.4 ± 0.8%	96.6 ± 0.3%	96.2 ± 0.8%
Radiochemical purity (HPLC)	97.9 ± 0.5%	93.0 ± 1.2%	91.7 ± 0.9%	94.2 ± 0.3%
Sterility	Sterile*	Sterile	Sterile	Sterile

*^{18}FDG was sterilized by membrane filtration (0.22 µm).

Results of sterility test demonstrated that all essayed conditions were able to ensure ^{18}FDG sterility. TLC method did not show any change in radiochemical purity of ^{18}FDG after steam sterilization. HPLC results evidenced a reduction in the radiochemical purity of ^{18}FDG after all autoclave cycles, probably caused by heat. These two methods were used to evaluate radiochemical purity of ^{18}FDG in order to cover both pharmacopeias [7, 9]. The European Pharmacopoeia [9] requires HPLC and TLC for the evaluation of radiochemical purity of ^{18}FDG, while the USP [7] requires TLC.

TLC method is considered accurate and reliable [10]; however, it was less sensitive than HPLC method for this analysis. No changes in TLC chromatograms were observed before and after steam sterilization, as exemplified by Fig. 2 and 3.

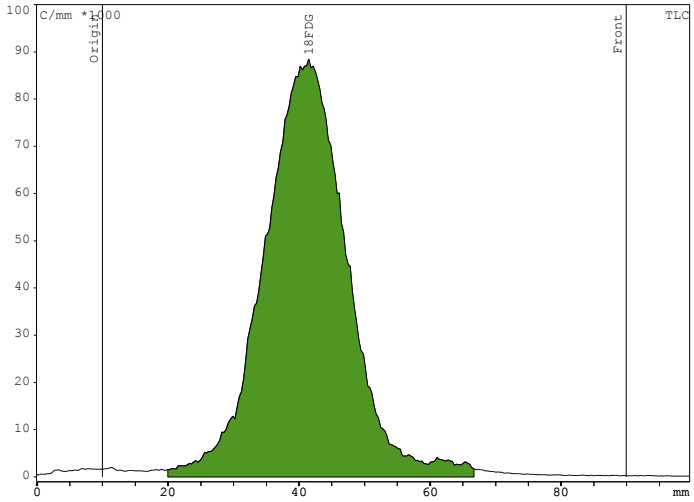

Figure 2. TLC chromatogram of ^{18}FDG before steam sterilization

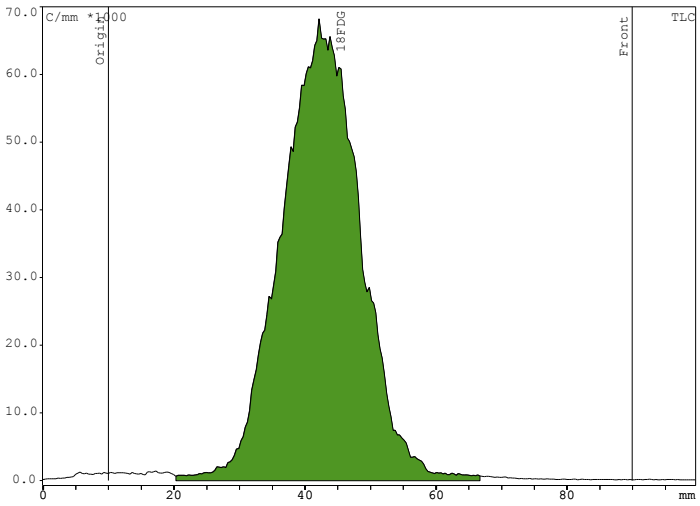

Figure 3. TLC chromatogram of ^{18}FDG after steam sterilization (133°C / 2 min)

HPLC method evidenced an enlargement of ^{18}FDG peak after all steam sterilization cycles, even in the less damaging condition (133°C/2 min), as shown in Fig. 4 and 5. A by-product seems to be formed after steam sterilization, possibly ^{18}Fludeoxymannose (^{18}FDM), though it is difficult to identify precisely this compound due to the poor resolution already reported for this method [10].

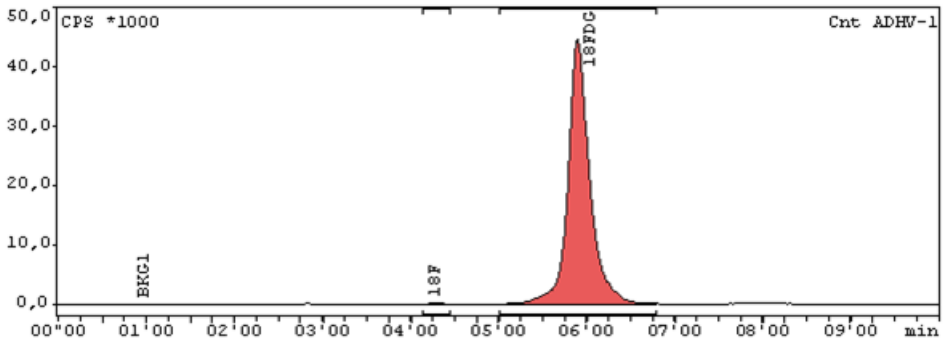

Figure 4. HPLC chromatogram of ^{18}FDG before steam sterilization

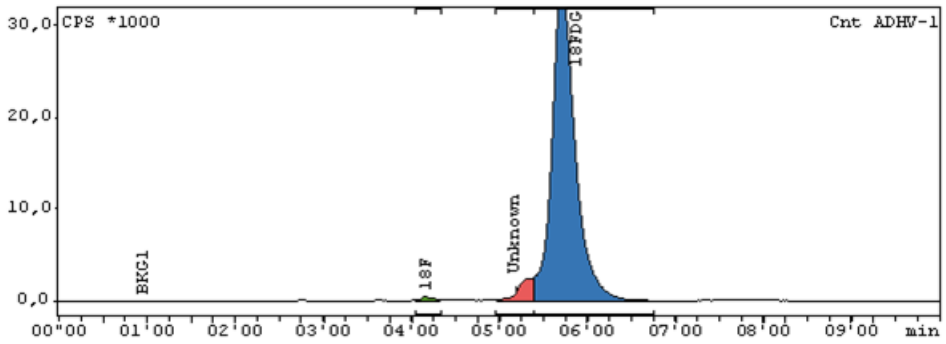

Figure 5. HPLC chromatogram of ^{18}FDG after steam sterilization (133°C / 2 min)

Previous studies [11, 12] have reported that ^{18}FDM can be formed as a result of base catalyzed epimerization of ^{18}FDG, which is time and temperature-dependent and occurs under alkaline conditions. Measureable epimerization was observed above room temperature in the presence of NaOH 0.3 $mol \cdot L^{-1}$, used in the hydrolysis of the intermediate 1,3,4,6-tetraacetyl-2-[^{18}F]fluoro-deoxy-D-glucose. In our study, pH-formulation was kept around 6.0 in order to minimize the formation of ^{18}FDM during the exposure of ^{18}FDG to higher temperatures. Although a reduction in the radiochemical purity of ^{18}FDG was observed after autoclave cycles, it was superior to the established limit (>90%) in all essayed conditions.

4. CONCLUSION

Steam sterilization was successfully applied to ^{18}FDG production, in compliance with GMP. The best essayed condition of sterilization was 133°C for 2 minutes. It was able to ensure sterility of the radiopharmaceutical and took less time. Although a reduction on radiochemical purity was observed after autoclave cycles, ^{18}FDG remained within the specifications.

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REFERENCES

1. W. Wadsak**,** M. Mitterhauser**, "**Basics and principles of radiopharmaceuticals for PET/CT", *European Journal of Radiology* **73,** 461-469 (2010).
2. P. Karwatha, J. Sartorb, W. Griesb, C. Wodarskib, C. Dittmarb, H. Biersacka, S. Guhlkea, "Steam sterilization and automatic dispensing of [18F]fludeoxyglucose (FDG) for injection", *Applied Radiation and Isotopes* **62**, 577–586 (2005).
3. European Commission, "Manufacture of sterile medicinal products", *Good manufacturing practice medicinal products for human and veterinary use*, **4** (2008).
4. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária, Diário Oficial [da] República Federativa do Brasil, Brasília, DF, **Resolução – RDC n°17, de 16 de abril de 2010**, (19 abr. 2010).
5. "Guide to good manufacturing practice for medicinal products" *Pharmaceutical inspection convention,* (2006).
6. Brasil. Agência Nacional de Vigilância Sanitária, Brasília, DF, Farmacopeia Brasileira, **5** (2010) (21 nov. 2010).
7. The United States Pharmacopeial Convention, *The United States Pharmacopeia 32*, U. S. Pharmacopeia, Rockville & USA (2009).
8. S. Ferreira, J. da Silva, S. Waquil, R. Correia, "Stability study of 2-[^{18}F]fluoro-2-deoxy-d-glucose (^{18}FDG) stored at room temperature by physicochemical and microbiological assays", *International Nuclear Atlantic Conference* - INAC 2009.
9. European Directorate for the Quality of Medicines & HealthCare, *European Pharmacopoeia 6.0,* European Directorate for the Quality of Medicines & HealthCare, Strasbourg & France (2008).

10. J. Hung, "Comparison of various requirements of the quality assurance procedures for ^{18}F-FDG injection", *J. Nucl Med*, **43,** pp. 1495-1506 (2002).
11. P .Varelis, R.K Barnes, "Epimerization of 2-Deoxy-2-[^{18}F]fluoro-D-glucose under basic conditions. A convenient method for the preparation of 2-Deoxy-2-[^{18}F]fluoro-D-mannose", *Applied Radiation Isotopes*, **47**, 731 – 733 (1996).
12. G. Meyer, K. Matzke, K. Hamacher, F. Füchtner, J. Steinbach, G. Notohamiprodjo, S. Zijlstra, "The stability of 2-[18F]fluoro-deoxy-D-glucose towards epimerization under alkaline conditions", *Applied Radiation Isotopes*, **51,** pp. 37 – 41 (1999).