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STUDY OF THE AUTOMATED SYNTHESIS OF THE RADIOPHARMACEUTICAL 16 α [^{18}F] FLUORO-17β-ESTRADIOL

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ABSTRACT

Approximately 75% of breast cancer cells express estrogen receptor (ER +) and this type of cancer has incidence of 25 % a year, being the second cause of cancer death among women worldwide. The 16α-[^{18}F]-fluoro-17β-estradiol, ^{18}F-FES, is a lipophilic molecule with *in vivo* characteristics similar to estradiol being a valuable marker in molecular imaging for non-invasive diagnosis of primary and metastatic breast cancer using PET-CT, because it binds to estrogen receptors (ER binding). The objective of this work was to study the synthesis of ^{18}F-FES in the GE TRACERlab® MX_{FDG} module, using Chemical Kit and disposable cassette ABX® and determine the yield of the process and the analytical parameters to be employed in the routinely production of this radiopharmaceutical. The automated synthesis occurs in 75 min. and includes percolation of the [^{18}F] fluoride in an anion exchange cartridge, elution of the cartridge, azeotropic drying in 3 steps, labeling using 3-methoxymethyl-16β,17β-epiestriol-O-cyclic sulfone (MMSE) and the hydrolysis in 1 step. Purification of the product is done in the module itself using solid phase extraction (SPE) cartridges, without the use of HPLC. The data about synthesis efficiency and quality control parameters are under analysis. Preliminary results suggest an increase in synthesis efficiency when minimal changes in the synthesis program were introduced. The results of quality control assays (radiochemical purity, residual solvent, radionuclidic purity and identity and chemical purity (TBA) suggest that final radiopharmaceutical meets the criteria established for other fluor-18 radiopharmaceuticals that have monographs in official compendia.

1. INTRODUCTION

Data from INCA, 2018 [1] show that approximately 75% of breast cancer cases present estrogen receptor (ER +) and this type of cancer has a growth incidence of 25% each year, besides being the second cause of death by cancer among women in the world. Currently, the technique of screening breast cancer considered gold standard in Brazil is mammography, however, it has some limitations: it is not recommended for women with dense breasts, women who have passed by treatment of radiotherapy and women with silicone prostheses. In these cases complementary tests in nuclear medicine are indicated, where the

radiopharmaceutical FDG-^{18}F (fludeoxyglucose-^{18}F) is widely used in the diagnosis, staging and to verify treatment efficacy in a wide range of tumors being a nonspecific tumor marker, since glucose metabolism is increased in tumor cells compared to non-tumor cells [3].

The 16α-[^{18}F]-fluoro-17β-estradiol (^{18}F-FES) is a lipophilic radiopharmaceutical with *in vivo* characteristics similar to estradiol and has high binding affinity for estrogen receptors. Studies have shown that ^{18}F-FES is a radiopharmaceutical of choice for investigating, by a noninvasive method, tumor size, disease sites, assists in the prognosis of individualized patient treatment, and can also be used for check for recurrent breast cancer and disease monitoring with hormone treatment [4]. The immunohistochemical (IHC) test is used in clinical routines to measure ER expression, but it is an invasive method. Peterson (2008), studying the uptake of ^{18}F-FES in breast cancer cells, demonstrated a positive correlation both qualitatively and semi-quantitatively between FES-^{18}F and IHC. This result demonstrated that ^{18}F-FES can be used to measure *in vivo* distribution, to quantify tumor burden and to detect metastases difficult to visualize in a biopsy [3.5]. When compared it presented 69-100 % sensitivity and 80-100 % specificity [6,7,2]. Kiesewetter et al. reported the first synthesis of ^{18}F-FES in 1984 [8] and then many articles were published using different types of synthesizer modules and synthetic routes.

This present work studied the synthesis of the ^{18}F-FES radiopharmaceutical in GE TracerLab MX module using a sequence program and ABX reagent kit (Germany) and evaluated the efficacy of analytical methods used in the quality control of the finished product.

2. METHODOLOGY

2.1 Radiosynthesis

The synthesis of ^{18}F-FES was based on the methodology described by Knott et al [9], using automated synthesis reagent kit (ABX, Germany) whose cassette assembly scheme is shown in figure 1. Some modifications were made in programming sequence aiming improve yield. The fluoride ($^{18}F^-$) was produced in the CYCLONE 18 MeV (IBA Cyclotron Solutions, Belgium), from the irradiation of enriched water ([^{18}O] H_2O) with protons. Upon entering the synthesis module, the irradiated water was percolated in anion exchange cartridge (QMA Light®) and then eluted to the reaction using 500µL of 0.075M tetrabutylammonium bicarbonate solution (TBA-HCO3). Then, two evaporations are performed and the water is then removed by azeotropic distillation with acetonitrile at 95 °C. The vial with 1.5 mg of the precursor (2-3-methoxymethyl-16β, 17β-epiestriol-O-cyclic sulfone - MMSE) was pre-diluted with 1.5 mL of acetonitrile and added to the anhydrous complex (^{18}F) + TBA, so followed by heating at 130 ° C for 5 min. After evaporation of the solvent, the temperature decreased for hydrolysis with 3 mL of sulfuric acid / ethanol solution in the reaction bottle. Hydrolysis occurs at 110 ° C for 5 min. After the hydrolysis, the reaction vial temperature droped to 40 ºC, and the reaction mixture was diluted with water and directed for purification in the different SPE cartridges (Oasis® WAX, Sep-Pak® C18, Oasis® HLB). These cartridges were washed slowly with 40% ethanol and the unwanted by products and the remaining precursor that have not been labeled were directed to the waste. The reaction vial was flushed with 40% ethanol, the ^{18}F-FES was eluted from the cartridges with 95% ethanol

and transferred back into the reactor vial at 90 ° C, 5 min for ethanol evaporation. Subsequently, the ^{18}F-FES solution was withdrawn from the reactor with 20 mL of water and purified on the Sep-Pak® Alumina-N cartridge and sterile filter 0.22µm. The total time of synthesis was approximately 75 min.

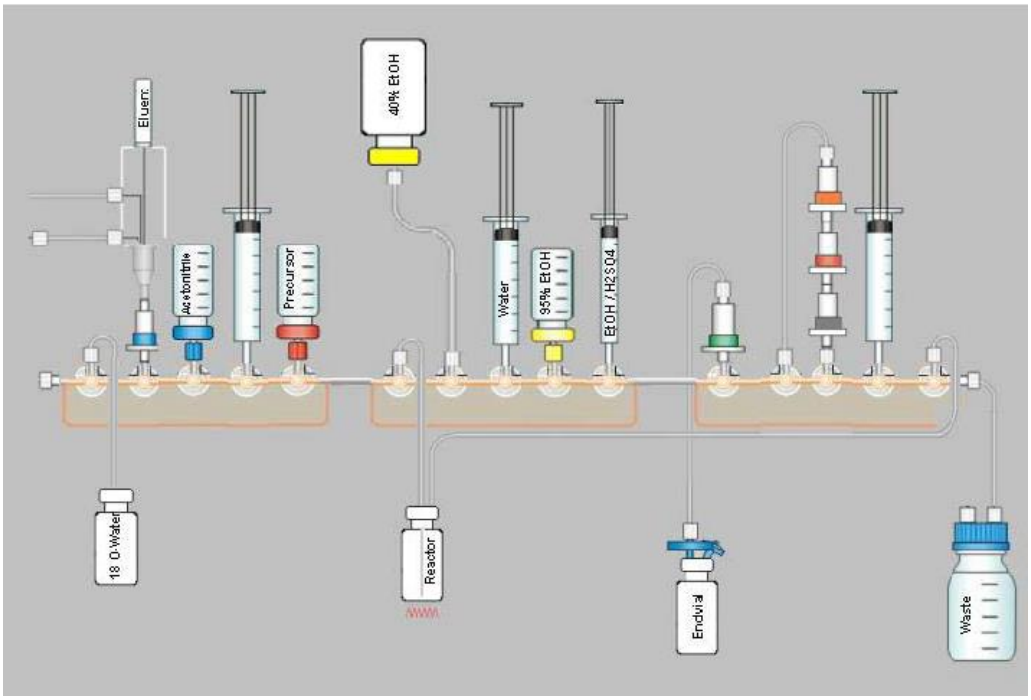

Figure 1 - General assembly of the ^{18}F-FES synthesis cassette in G.E TracerLab MX module

2.2 Quality Control

Radionuclide identification consisted in the determination of the half-life using a Dose Calibrator (Capintec, USA); the FES-^{18}F sample activity was measured three times every 10 minutes (30 minutes). The half-life obtained was compared with the adopted specification (105 to 115 minutes), with the fluoride-^{18}F half-life of 109.7 minutes [10].

Radiochemical purity was performed by HPLC (Shimadzu, USA), according to the method described by Bishop et al. 2017 [11] with modifications: Column C18 (5µm, 250 x 4.6mm) eluted with 1mL / min acetonitrile:water 40:60 (v / v) with radioactivity and UV sensor (280 nm) and injection volume 50µL. Radiochemical Purity by Thin Layer Chromatography was performed using silica gel 60 (TLC-SG) coated aluminum strips as stationary phase and 95% acetonitrile as mobile phase [5,12] and the radioactivity was detected with Gamma Counter (Packard, USA). The obtained results were confronted with the acceptance criterion ≥ 95% [10,14].

Determination of the residual solvent, acetonitrile, was done using gas chromatography (GC) (Shimadzu, USA). The conditions required for the analysis were furnace temperature at 85 °C, transfer line temperature at 115 °C, loop temperature at 100 °C, 15 minute bottle equilibration time, supply loop time of 0.2 minutes, 1.0 minute injection time, 0.2 minute pressurizing time, 0.5 minute loop equilibrium time. The column was initialized at 40 °C and then increasing 2 °C/min until reaching a temperature of 50 °C. The injector temperature and detector were 250 °C, for both [5,12]. The results obtained were compared with the specification presented in the American Pharmacopoeia, fludesoxyglucose ^{18}F-FDG monogra ph [14], described by Feltes 2011 (No more than 0.04% acetonitrile) and in the Draf of the FES-^{18}F, 2017 (Acetonitrile < 410 ppm).

The pH was determined with indicator strips, with a range between 0 and 14. The values obtained were compared with the specification presented in the American Pharmacopoeia, fludesoxyglucose ^{18}F-FDG monograph [14], of 4.5 - 7.5, described by Feltes 2011 (4.5 - 8.5) and the Draft of the FES-18F [13] (4.5 -8.0).

3. RESULTS AND DISCUSSION

Three syntheses were performed, the average activity of enriched water irradiated ([^{18}F] H_2F^+) measured at the entrance in the module was 1.48 × 1014 Bq (4.0 Ci). Labeling Yield was 11.0 to 13.3 % (non-decay corrected), the whole process which takes 75min.

The results obtained in the tests carried out in the three lots are shown in Table 1. The values are average of three determinations and fully meet the specification.

Table 1: Results of the parameters analyzed in the quality control and the specification presented in the American Pharmacopoeia, fludesoxyglucose ^{18}F-FDG monograph.

Labeling	1	2	3	Specification USP
Labeling Yield (%)	13.3	11.0	12.0	10 - 20%
Radiochemical purity* (%)	98.8	99.0	99.0	≥ 95%
Radionuclidic identity (minutes)	110.0	110.0	110.0	105 - 115
Acetonitrile (ppm)	0.0	n.t**	0.0	< 410 ppm
pH	7.0	6.5	7.0	4.0 - 7.5

* TLC-SG assay

** No tested

Figure 2 shows the TLC-SG profile of the fluoride [^{18}F] and final product ^{18}F-FES demonstrating the separation ability of the system employed.

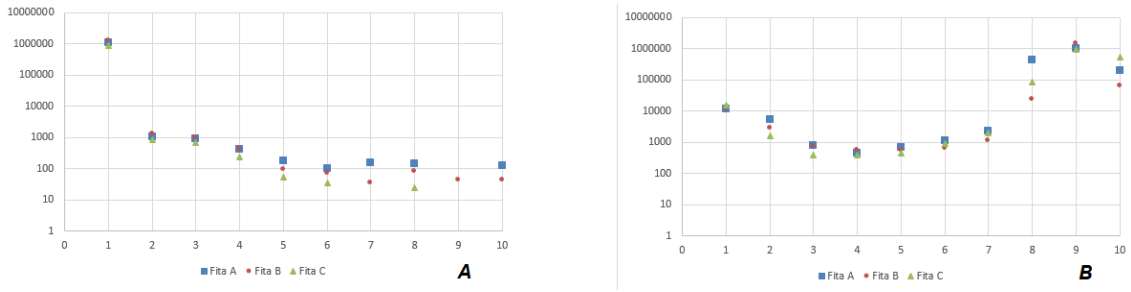

Figure 2 – (A) Chromatographic profile of ^{18}F and (B) Chromatographic profile of FES-^{18}F in TLC-SG

The HPLC chromatographic profile of ^{18}F-FES (Figure 3), shows the good resolution of ^{18}F-FES peak. The retention time obtained (between 15 and 17 minutes) is in agreement with that expected for a hydrophilic species, considering the reverse phase system used and is compatible with the literature [9,11].

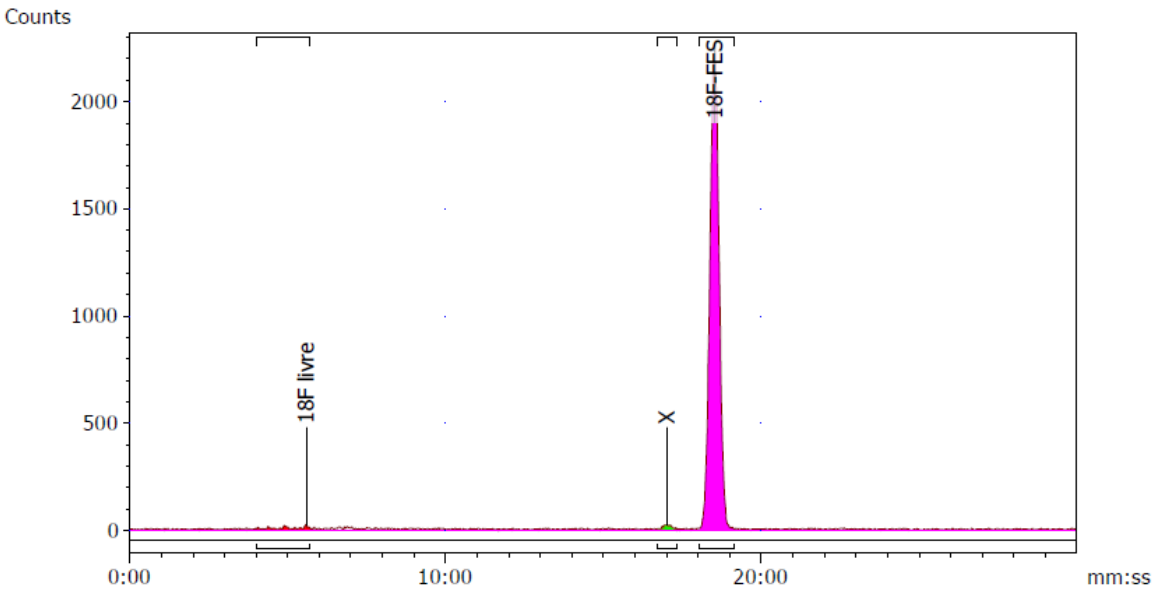

Figure 3 - HPLC radiochromatogram of FES-^{18}F

The residual acetonitrile was below the value detected by the equipment, reporting as 0.00 mg/mL.

The methods used in the quality control trials have proved to be suitable for the evaluation of the finished product ^{18}F-FES and will be validated since this radiopharmaceutical do not has Pharmacopoeia monograph.

In addition, the assay for determination of TBA-HCO^3 residue will be developed, in the

continuity of this work, as well as biodistribution sdudies of ^{18}F-FES in animal model with breast tumor.

4. CONCLUSIONS

In this work, we have studied the automated system for the production of the ^{18}F-FES radiopharmaceutical in GE TRACERlab ™ MXFDG module that demonstrated to be easy to use, reliable and reproducible; the ease of non-use of HPLC for purification of the final product, since, purification is done on the module itself using a combination of different single-use SPE cartridges, makes this synthesis more practical and faster, 75 minutes. We obtained low radiochemical yield of 13.0 %, in agreement with the literature, but the QC results showed that the product met all the specifications found in accordance with USP and FDA radiopharmaceutical guidelines.

The development of the ^{18}F-FES radiopharmaceutical at IPEN / Brazil is of great interest to offer the Brazilian nuclear medical class a new radiopharmaceutical for use in PET and CT-PET, assisting in the diagnosis and follow-up of patients with breast cancer.

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