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Synthesis and characterization of the radiopharmaceutical [^{18}F]fluoroestradiol

Bispo[a] A.C.A., Nascimento[a] L.T.C., Castro[a] A.C.F., Lima[a] L.A.R., Ferreira[a] S.M.Z.M.D., Silva[a] J.B., Mamede[b] M.

[a] *Unidade de Pesquisa e Produção de Radiofármacos/Centro de Desenvolvimento da Tecnologia Nuclear (CDTN / CNEN – MG), 31270-901, Belo Horizonte, MG, Brazil*

[b] *Departamento de Anatomia e Imagem/Faculdade de Medicina/Universidade Federal de Minas Gerais (UFMG), 30130-100, Belo Horizonte, MG, Brazil*

anacarollbispo@gmail.com

ABSTRACT

[^{18}F]Fluoroestradiol ([^{18}F]FES), an estrogen analog, may be used in Positron Emission Tomography (PET) for evaluation of the tumor cell receptor profile in a noninvasive way, which is an important factor for determining the therapy to be used, disease staging, prognosis and response to therapy in breast cancer. [^{18}F]FES is a new radiopharmaceutical and does not have an official monograph in any pharmacopeia until now. Therefore, the objective of this work was the optimization of the [^{18}F]FES automatic synthesis and the elaboration of an analytical protocol for final product analyses. Initially, the synthesis of [^{18}F]FES was performed according to a modified commercial protocol, using a TracerLab MX$_{FDG}$ synthesis module specific for [^{18}F]FES synthesis. New protocols for the physicochemical quality control assays of the radiopharmaceutical were developed, including pH, chemical purity, residual solvents, radiochemical identity and purity. Physicochemical and microbiological analysis were performed with the synthesized [^{18}F]FES at different time points to evaluate its stability. The uncorrected synthesis yield was 18.37 ± 3.07 %. All Quality Control methodologies proved to be effective for the product. The physicochemical and microbiological results obtained in the stability study showed that [^{18}F]FES is stable during 8 hours after its synthesis, even under stress conditions. At the end of the study, it was concluded that the [^{18}F]FES complies with all specifications required for a radiopharmaceutical intended for diagnostic use.

Keywords: Radiopharmaceuticals, Breast Neoplasms, Product Synthesis, Quality Control.

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1. INTRODUCTION

Breast cancer is the most common cancer in the world. If diagnosed and treated early, breast cancer has a good prognosis. However, mortality rates from this cancer remain high in Brazil, most likely because the disease is still diagnosed in advanced stages [1].

On the other hand, Positron Emission Tomography (PET) may be used to identify biological and functional changes that occur in tumor cells, thus generating an early diagnosis of cancer in a noninvasive way. [^{18}F]Fludeoxyglucose ([^{18}F]FDG), which is based on the increased glycolytic metabolism of tumor cells [2], is the most widely radiopharmaceutical used for breast cancer diagnosis. However, it is known that, biologically, this radiopharmaceutical may not be the ideal radiotracer to detect this type of cancer [3]. Infection and inflammation sites may generate false positive results, whereas malignant tumors with low metabolic activity may result in false negatives [4]. Thus, to optimize the detection of neoplastic cells, it is necessary to develop new radiopharmaceuticals directed at other tumor markers.

Breast cancer is a pathology that presents several subtypes, which are differentiated by the expression profile of hormone receptors in tumor cells. Approximately two-thirds of breast cancers are estrogen-dependent, progesterone-dependent or both [5]. Thus, the development of a radiopharmaceutical that has affinity for these receptors is important.

The use of [^{18}F]Fluoroestradiol ([^{18}F]FES), an estrogen analogue, allows the evaluation of the receptor profile of tumor cells, an important factor for determining the therapy to be used, with potential value in disease staging, prognosis and response to the therapy [2,6].

Currently, several methodologies for production and quality control of [^{18}F]FES are found in the literature. However, an optimal synthesis for routine production should be completed in the shortest possible time, with high yield and the lowest possible cost. The methodologies used for quality control must generate the necessary information quickly, reliably and linearly, as well as having the lowest possible cost. Therefore, experiments were done to optimize the automatic synthesis and to determine an analytical protocol for the final product, aiming to achieve methodologies with the characteristics described.

2. MATERIALS AND METHODS

2.1. Reagents and equipment

For [^{18}F]fluoride radionuclide production were used: Cyclotron PETrace 16.5 MeV from General Eletric Healthcare (UK); enriched ^{18}O water from the Center of Molecular Researches Isotopes (Russia).

For synthesis were used: Automatic Synthesis Module TracerLab MX_{FDG} from General Electric Healthcare (UK); cassette and kit for [^{18}F]FES synthesis from ABX (Germany).

To perform the [^{18}F]FES quality control and stability tests were used: FES standard and precursor 3-O-methoxymethyl-16,17-O-sulfuryl-16-epiestriol (MMSE) standard from ABX (Germany); High performance liquid chromatography (HPLC) grade acetonitrile, absolute ethanol, Kryptofix® 2.2.2 standard, Tetrabutylammonium (TBA) standard, pH 0-14 tapes from Merck (Germany); Perkin-Elmer Gaseous Chromatography equipment model Clarus 680 (USA), equipped with flame ionization detector (FID) and integrated liquid autosampler, with DB-WAX fused silica capillary column, size 30 m × 0.25 mm size, with 0.5 μm film thickness, from Agilent Technologies (USA); Agilent Technologies HPLC equipment model 1200, with 20 μL hand injector and loop, equipped with Luna C18 (2), 5 μm, 250 x 4.6 mm column (Phenomenex, USA), radioactivity and ultraviolet detectors; dose calibrator CRC-25R from Capintec Inc. (USA); Multichannel Analyzer with High Purity Germanium (HPGe) detector from Canberra Industries (USA); pHmeter S20 from MettlerToledo (Switzerland).

2.2. Synthesis of [^{18}F]fluoroestradiol

[^{18}F]fluoride was produced via the ^{18}O(p,n)^{18}F nuclear reaction in a Cyclotron and [^{18}F]FES synthesis was performed in automatic synthesis module. The methodology was based on Knott *et al* [7]. Considering other previously published synthesis protocols [8-15], 4 parameters were varied in order to increase the radiochemical yield of [^{18}F]FES: a) the phase catalyst (Kryptofix® 2.2.2 (0.053 mmol) and tetrabutylammonium hydrogen carbonate (0.056 mmol)); b) the labeling reaction temperature (110 °C and 130 °C); c) the hydrolysis reaction temperature (110 °C and 130 °C); d)

the transfer of the radiopharmaceutical produced solution to the final flask (partial and total transfer).

2.3. Quality control of [^{18}F]fluoroestradiol

The physicochemical quality control assays included pH, chemical purity, residual solvents (ethanol and acetonitrile), radionuclidic identity and purity and radiochemical identity and purity. The methodologies used in the assays of [^{18}F]FES were based on the work of Kuntzsch *et al* [16] (chemical purity), the work of Bispo *et al* [17] (radiochemical identity and purity), the techniques used for [^{18}F]fluorocholine analyses (pH assay) and for [^{18}F]FDG analysis (residual solvents, radionuclidic identity and purity) described in the US Pharmacopeia [18].

2.4. Stability of [^{18}F]fluoroestradiol

Stability of three different batches of [^{18}F]FES produced in a maximum radioactive concentration (2.4 GBq·mL^{-1}) was evaluated by physicochemical and microbiological assays. The radiopharmaceutical was kept in its primary package and in tungsten radiation shielding container. [^{18}F]FES was maintained under controlled temperature (40 ± 2 °C) and humidity (75 ± 5%) within a climatic chamber throughout the stability study. Fifteen perforations were made in the cap of the primary package, simulating the real use of the product. All physicochemical and microbiological quality control tests were performed at 2-hour intervals immediately after end of synthesis, for up to 8 hours. The methodologies used in the microbiological analysis of [^{18}F]FES were based on the techniques used for [^{18}F]FDG microbiological analysis described in the US Pharmacopeia [18].

3. RESULTS AND DISCUSSION

3.1. Synthesis of [^{18}F]fluoroestradiol

The [^{18}F]Fluoroestradiol synthesis was performed according the original ABX's synthesis protocol, published by Knott *et al* [7]. [^{18}F]FES was obtained with radiochemical yield of 10.54%, decay-uncorrected relative to starting [18F]fluoride. This radiochemical yield obtained did not correspond to the radiochemical yield described for Knott *et al* [7] and other authors [8-15] (decay-

uncorrected radiochemical yield of 20%). In order to increase the radiochemical yield of [^{18}F]FES, the phase catalyst, the labeling reaction temperature, the hydrolysis reaction temperature, and the transfer of the radiopharmaceutical produced solution to the final flask were varied. The decay-uncorrected radiochemical yield for each variation is shown in Table 1.

Table 1: *Variations made in the [^{18}F]FES synthesis protocol and the decay-uncorrected radiochemical yields obtained. The final synthesis protocol established was Variation 4.*

	Phase Catalyst	Labeling Temp.	Hydrolysis Temp.	Transfer Volume	Decay-uncorrected Radiochemical Yield	
					Average	SD
Original protocol	TBA-HCO_3	130 °C	110 °C	Partial	10.54%	1.39%
Variation 1	Kryptofix® 2.2.2	130 °C	110 °C	Partial	0.17%	0.12%
Variation 2	TBA-HCO_3	130 °C	130 °C	Partial	8.64%	1.05%
Variation 3	TBA-HCO_3	110 °C	110 °C	Partial	11.00%	1.07%
Variation 4	TBA-HCO_3	110 °C	110 °C	Total	22.12%	2.17%

The final synthesis protocol established uses as the phase catalyst tetrabutylammonium hydrogen carbonate, both labeling reaction and hydrolysis reaction temperatures at 110 °C, and complete transfer of the radiopharmaceutical solution to final flask (Variation 4).

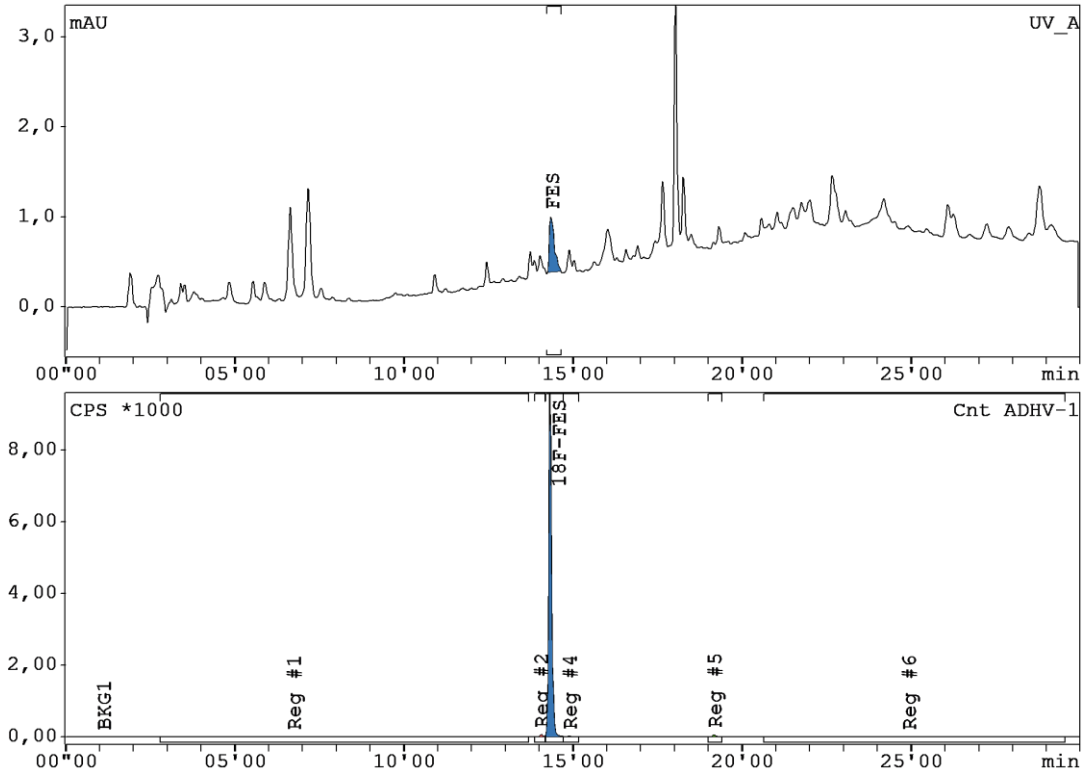
The mean synthesis time was about 75 min. [^{18}F]FES was obtained in a 10% w/v ethanolic saline solution, with a final volume of 15 mL.

3.2. Quality control of [^{18}F]fluoroestradiol

The [^{18}F]FES does not have official monograph in any recognized Pharmacopeia. Therefore, to define the physicochemical characterization tests, the official monographs of other radiopharmaceuticals and published works were used. The residual solvents, radionuclidic identity and purity were performed as described in [^{18}F]Fludeoxyglucose official monograph [18]. The pH test was performed as described in [^{18}F]Fluorocholine official monograph [18]. The chemical purity test, which determines the concentration of TBA-HCO_3 used as phase catalyst, was performed as described by Kuntzsch *et al* [16]. The radiochemical identity and purity tests were based on work of Bispo *et al* [17] with modifications in order to improve the peaks resolution for [^{18}F]FES.

In the analytical protocol established for radiochemical identity and purity, the analysis was performed in liquid chromatograph equipped with an ultraviolet detector at 280 nm, an radioactive detector, and a stationary phase C18(2) column with 5 µm particle size, 4.6 x 250 mm. Elution was performed with mobile phase acetonitrile (ACN) in water, with flow of 1.2 mL·min^{-1}. The concentration of the mobile phase ACN:water was variated during the run as follows: 0' to 5' – 30:70 isocratic; 5' to 20' – gradient of 30:70 to 90:10; 20' to 30' – 90:10 isocratic. The total run time was 30 minutes. The [^{18}F]FES retention time was 14.32 minutes and the radiochemical purity achieved was 97.00% (Figure 1).

Figure 1: *HPLC chromatogram of [^{18}F]FES radiochemical identity and purity analysis. Conditions: mobile phase acetonitrile:water 0' to 5' – 30:70 isocratic; 5' to 20' – gradient of 30:70 to 90:10; 20' to 30' – 90:10 isocratic at 1.2 mL·min^{-1}; stationary phase C18(2) column with 5 µm particle size, 4.6 x 250 mm; radioactivity (lower chromatogram) and UV (upper chromatogram at 280 nm) detectors. Radiochemical purity: 97.00 %.*



The product of the syntheses performed with the chosen protocol (Variation 4) was subjected to all the quality control tests described. The product was approved in all tests, with results within the

range considered as ideal in recognized pharmacopeias. The average results obtained for each characterization test are presented in Table 2. Results indicate that the synthesized product follows specifications, corroborating the chosen synthesis protocol.

Table 2: *Mean of the quality control results for the synthesis of the [^{18}F]FES produced and their respective limits (n = 3).*

Tests	Results	Specified Limits
pH	5.68	4.5 – 8.0
Chemical Purity - TBA-HCO_3	< 50 µg·mL^{-1}	< 150 µg·mL^{-1}
Radionuclidic Identity	110.3 min	105 – 115 min
Radionuclidic Purity	99.96%	≥ 99.5%
Radiochemical Purity	94.3%	> 90%
Acetonitrile Concentration	0.0039%	< 0.04%
Ethanol Concentration	4.93%	< 10%

3.3. Stability of [^{18}F]fluoroestradiol

All physicochemical and microbiological test results (not shown) for the three batches of [^{18}F]FES at 0; 2; 4; 6 and 8 hours after the synthesis were in accordance with the specified limits. These results demonstrate that the radiopharmaceutical remains stable up to 8 hours after the synthesis at the maximum radioactive concentration, even under critical conditions of temperature and humidity (40 ± 2 °C / 75 ± 5% relative humidity). No influence was observed for the perforations made in the cap of the primary package on the quality requirements of this radiopharmaceutical.

4. CONCLUSION

The ABX synthesis protocol for [^{18}F]FES, based on the work by Knott *et al* [7], was ineffective in achieving the mean yields of 20% presented by several authors [8-15]. Therefore, changes were made in the temperature of the labeling reaction and the volume transferred from the reactor solution, which were shown to be essential to reach the average yield described in the literature.

The methodologies applied to the analysis of [^{18}F]FES in the quality control were effective.

The radiopharmaceutical remains stable up to 8 hours after the synthesis at the maximum radioactive concentration (2400 $MBq \cdot mL^{-1}$), even under critical conditions of temperature and humidity.

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