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Development of an HPLC method for the radiochemical purity evaluation of [^{18}F]fluoroestradiol

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ABSTRACT

^{18}F-Fluoroestradiol ([^{18}F]FES), an estrogen analog, is a radiopharmaceutical used in Positron Emission Tomography (PET) that allows evaluating the tumor cell receptor profile and the best therapy strategy, the staging, the prognosis and the response to therapy in several breast cancer cases. As there is not any pharmacopoeia's monograph of [^{18}F]FES to standardize its quality control criteria, this work presents a new HPLC's method to perform the [^{18}F]FES radiochemical purity. A liquid chromatograph was used with radioactivity and ultraviolet detectors. Three concentrations of fluoroestradiol standard solution were used along the test. Their retention time was compared to its relative radiolabelled analogue to confirm its identity. Several mobile phases with acetonitrile and two mobile phase flows were tested to optimize the runs. Peaks symmetry, retention time, theoretical plates and resolution were analyzed to choose the best conditions. The mean retention time of both standard Fluoroestradiol and [^{18}F]FES solutions were the same, demonstrating that [^{18}F]FES formulation did not interfere with [^{18}F]FES analysis. The best conditions were 1.2 mL/min and isocratic 40% V/V acetonitrile in water, which gave [^{18}F]FES peak resolution greater than 6 and symmetry factor of 1. Thus, the developed method is ready to be validated and implemented in [^{18}F]FES quality control routine in CDTN/Brazil.

Keywords: ^{18}F-Fluoroestradiol, HPLC, Radiochemical Purity.

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1. INTRODUCTION

Breast cancer is the most prevalent type of cancer in women and the principal cause of death by cancer worldwide [1]. If diagnosed and treated early, breast cancer has a good prognosis. However, mortality rates from this cancer remain high in Brazil, most likely because this disease is still diagnosed in advanced stages [2]. An early diagnosis allows the treatment to be started more quickly, which has an important impact in reducing the mortality and the morbidity of the disease [3].

Positron Emission Tomography (PET) is an exam that allows the molecular evaluation of physiological, biochemical and pharmacological events that occur in a cell [4]. It can be used to identify the biological and functional changes that occur in a tumor cell, generating an early diagnosis of cancer in a noninvasive way. Currently, 18F-fludesoxyglucose ([^{18}F]FDG) is the most widely used radiopharmaceutical for breast cancer diagnosis, and its use is based on the affinity for cells with increased glycolytic metabolism [5]. However, it is known that it may not be the ideal radiotracer for the image of this type of cancer [6]. Infection sites may generate false positive results, whereas malignant tumors with low metabolic activity may result in false negative results [7]. Thus, to optimize the detection of neoplastic cells, it is necessary to develop new radiopharmaceuticals directed to other tumor markers.

Breast cancer is a pathology that presents several subtypes, which are differentiated by the expression profile of hormone receptors. Approximately two-thirds of breast cancers have estrogen-dependent and/or progesterone-dependent growth [8]. Thus, the development of a radiopharmaceutical that has affinity for these receptors is desirable. Once the [^{18}F]fluoroestradiol ([^{18}F]FES), an estrogen analogue, has been considered a promising radiotracer for breast cancer cases, its use has been proposed in this study [9].

[^{18}F]Fluoroestradiol has similar characteristics to estradiol, both in its molecular structure and in its transport in the organism, providing high affinity for nuclear estrogen receptors [9]. Its use in the clinic allows the evaluation of the receptor profile of tumor cells, an important factor for determining the best therapy to be used, with a potential value in the staging of the disease, in the prognosis and in the response to the therapy used [5,9].

[^{18}F]FES does not have an official monograph in any Pharmacopeia. Thus, all quality control tests must be developed and validated by its producers. One of these tests is the radiochemical purity, which measures the percentage of the radiopharmaceuticals's activity in the entire formulation's activity. This analysis is also used to determine the efficiency of the labeling step of the molecule with ^{18}F [10]. Therefore, the objective is validate a new methodology for the analysis of the radiochemical purity of [^{18}F]fluoroestradiol.

2. MATERIALS AND METHODS

2.1. Materials

Fluoroestradiol and precursor (MMSE) standards were purchased from ABX (Germany). Acetonitrile HPLC grade was purchased from Merck (Germany). Water was purified in a Milli-Q Plus system (Millipore, USA). The cassette, reagents kit and software sequence used for [^{18}F]FES synthesis were purchased from ABX (Germany). ^{18}O enriched water used to ^{18}F production was purchased from Center of Molecular Researches Isotopes (Russia)

2.2. Instrumentation

A General Eletric TracerLab MXFDG module (UK) was used for the [^{18}F]FES synthesis. ^{18}F was produced in a 16,5 MeV GE Cyclotron PETrace (UK).

An Agilent Technologies HPLC (High performance liquid chromatography) system, model 1200 (USA), was used for method development. The HPLC system was equipped with a manual injector, 20 µL loop, radiation (Raytest, Germany) and ultraviolet (Agilent Tecnologies, at 280 nm) detectors. Data acquisition, analysis, and reporting were performed using the equipment software. HPLC analysis was conducted using a Luna C18 (2) column (Phemonenex, USA), with 5 µm particle size, 4.6 mm internal diameter and 250 mm length.

2.3. Synthesis of [^{18}F]Fluoroestradiol

[^{18}F]FES synthesis was based on the methodology described by Knott et al [11], but some modifications were made aiming to improve yield. [^{18}F]Fluoride was produced via the ^{18}O(p,n)^{18}F

nuclear reaction. Both fluorination and hydrolyses steps were performed at 110 °C. [^{18}F]FES is obtained in a 10% w/v ethanolic saline solution sterilized through a 0.22 μm membrane filter. Total synthesis time was about 75 min.

2.4. Preparation of Standard and Sample Solutions

A stock solution of 100 μg/mL of fluoroestradiol (reference standard for 16α-[^{18}F]Fluoroestradiol, ABX, Germany) was prepared in acetonitrile (Merck, Germany) and subsequent dilutions were performed to obtain standard solutions of 1.0, 5.0 and 10.0 μg/mL. Similarly, 3 standard solutions, with same concentrations, were obtained for MMSE (precursor for 16α-[^{18}F]Fluoroestradiol, ABX, Germany), similarly diluted in acetonitrile. The standards and samples had previously been filtered through a 0.22 μm pore size filter (Millipore, USA) prior to injection.

The [^{18}F]FES samples were obtained directly from the final solution produced, without dilution.

2.5. Chromatographic Conditions

Chromatographic analyses were performed with a sample injection volume of 20 μL and the detection wavelength in 280 nm. The mobile phase consisted of isocratics mixtures acetonitrile:water, in the proportions 40:60 (v/v), 50:50 (v/v), 60:40 (v/v), 70:30 (v/v) or 80:20 (v/v). Two gradients conditions were also tested: one ranging from 70% (v/v) to 90% (v/v) and another ranging from 80% (v/v) to 90% (v/v) of acetonitrile. Flow rates of 1.0 and 1.2 mL/min were tested, aiming to improve the runs. The method run time was 30 min, and all experiments were performed at 20 °C. Peaks symmetry, retention time (t_R), theoretical plates (N) and resolution (R_s) were obtained through the chromatograph software and analyzed to choose the best conditions. Symmetry was calculated by the formula $A_s = w_{0.05}/2d$, where $w_{0.05}$ is the width of the peak at 5% height and d is the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline [12,13]; Theoretical plates was calculate by $N = 5.54\ (t_R/w_{1/2})^2$, where $w_{1/2}$ is the peak width at half height [12,13]; and resolution by the formula $R_s = (\sqrt{N})/4 \times (\alpha-1)/\alpha \times k/(k+1)$, where α is the separation factor and k is the retention factor [12].

3. RESULTS AND DISCUSSION

During the analyses, an instable baseline, peaks with shoulders and an irregular shape of the [^{18}F]FES peak were observed when the acetonitrile proportion was higher than the water. Increasing the water proportion in the mobile phase, the peaks in the chromatogram have become each more regular and with higher resolution. Acetonitrile:water proportion of 40:60 (v/v) gave peaks properly spaced in chromatogram with satisfactory values of symmetry, theoretical plates, resolution (Table 1) and a stable baseline. This mobile phase was the best [^{18}F]FES analysis condition, which is in agreement with previous work [14,15].

Table 1: Chromatographic parameters of [^{18}F]FES peak, when analyzed with the mobile phase acetonitrile:water 40:60 (v/v) and 1.2 mL/min.

Parameters	UV detector[a]	Radiation detector[a]	Reference
Resolution	6.35	1.15	> 1 [12]
Symmetry	0.941	1.010	As close as possible to 1.0 [12,16]
Theoretical plates	15186	13285	Highest possible value [12,13,16]
Retention time	13.7	13.7	-

a. Average results (n = 3).

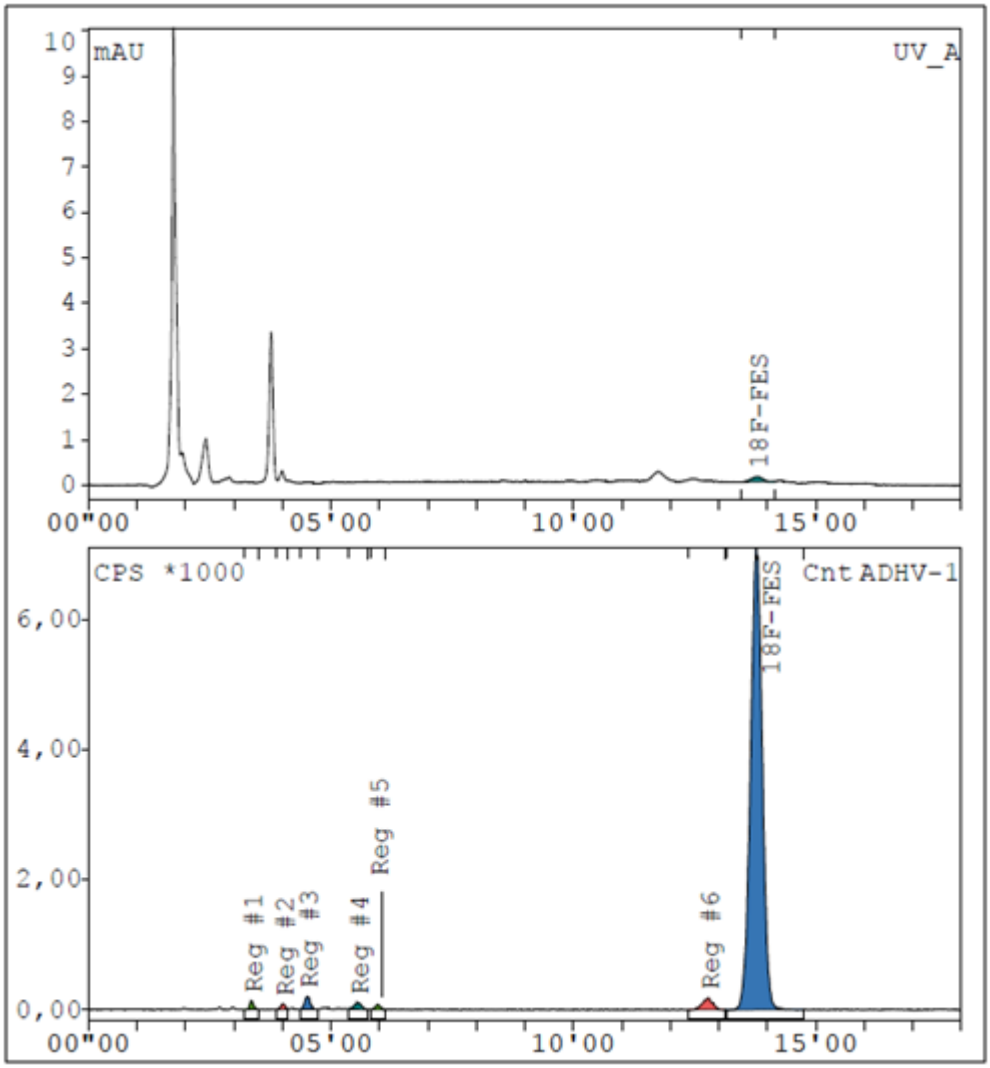
In order to optimize the analysis time of [^{18}F]FES, the flow from the mobile phase was varied from 1.0 mL/min to 1.2 mL/min. The flow rate of 1.2 mL/min caused a shift in the peaks, leading to a decrement in the retention time of all peaks. Therefore, the total run time could be reduced from 30 to 18 minutes. Under these conditions, the [^{18}F]FES retention time was 13.7 min (Figure 1) and the mean retention time of both standard fluoroestradiol and [^{18}F]FES solutions were the same (Table 1), demonstrating that [^{18}F]FES formulation, did not interfere with HPLC analysis.

It was also observed that there is no precursor's peak in the [^{18}F]FES formulation chromatogram, suggesting that is fully consumed or degraded. This fact is reported in a previous work [17].

Some impurities were observed in the final products chromatograms, although not identified yet. However, the sum of these peaks areas did not exceed the impurities limit of 10% presented in the

American Pharmacopoeia, in [^{18}F]FDG monograph [13], demonstrating that the observed impurities do not interfere with the final product quality.

Figure 1: *HPLC chromatogram of [^{18}F]FES. Conditions: mobile phase acetonitrile:water 40:60 (v/v) at 1.2 mL/min; 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. Injected activity: 1,3 mCi. Radiochemistry purity: 94.41%.*



4. CONCLUSION

The best condition to the analysis of [^{18}F]FES radiochemistry purity was 1.2 mL/min and isocratic 40% V/V acetonitrile in water, which gave [^{18}F]FES peak resolution greater than 1 and symmetry factor of 1. This method proved to quantify [^{18}F]FES independently of formulation components, as well as prove that precursor is not detected in the final product. In addition, this methodology proved to be effective, being able to identify degradations and formulation changes when present.

Thus, the developed method is ready to be validated and implemented in [^{18}F]FES quality control routine in CDTN/Brazil.

5. ACKNOWLEDGMENT

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