Published in final edited form as:

J Labelled Comp Radiopharm. 2014 December ; 57(14): 730–736. doi:10.1002/jlcr.3248.

Rapid synthesis of [^{18}F]-fluoroestradiol: remarkable advantage of microwaving over conventional heating

Jianfeng Shi, George Afari, and Sibaprasad Bhattacharyya*

ADRD, Frederick National Laboratory for Cancer Research (FNLCR), Leidos Biomedical Research, Frederick, MD, 21702

Abstract

16α -[^{18}F]-fluoroestradiol ([^{18}F]FES) is known as a clinically important tracer in nuclear medicine as an estrogen receptor (ER) ligand for investigating primary and metastatic breast cancers. Synthesizing [^{18}F]FES is a two-step process associated with [^{18}F]fluoride incorporation to the precursor (3-methoxymethyl 16β , 17β -epiestriol-O-cyclic sulfone, MMSE) and subsequent hydrolysis of the [^{18}F]fluorinated intermediate with 2N HCl. The impact of microwave heating (MW) on both fluorination and hydrolysis reactions was investigated. The duration and temperatures of the fluorination reaction were varied for both MW and conventional heating (CH) methods. Chemical and radiochemical purities and radiochemical yields were investigated for CH and compared to microwave-assisted radiosyntheses. Quality control tests of microwave-assisted [^{18}F]FES were performed following United States Pharmacopeia (USP) procedures for clinical-grade positron emission tomography (PET) pharmaceuticals. The results demonstrate that microwaving not only improves the ^{18}F -fluoride incorporation (~ 55% improvement at 110 °C for 4 min) but also significantly reduces hydrolysis time (~ 7-fold reduction at 120 °C) in compare to CH under similar conditions. The overall isolated radiochemical yield of purified [^{18}F]FES was significantly higher (~ 90% improvement) with MW, and side-products were notably fewer. Quality control test results demonstrated that [^{18}F]FES produced by microwaving was suitable for human injection.

1. Introduction

Although microwave dielectric heating has been used to speed-up the chemical transformations in common organic synthesis laboratories for a few decades,¹ this heating technique remains underutilized in the field of PET radio-tracer synthesis. The biggest challenge in radio-tracer synthesis with short lived isotopes (^{18}F and ^{11}C) is how to store the product for future use.² Therefore, it is extremely important that radiotracers are synthesized with high radiochemical yield to last long enough to carry out imaging studies. It has been proven that MW not only increases the product yield, but also shortens the reaction time, increases reproducibility, and produces a cleaner product mixture when compared to conventional heating. Realizing these advantages, microwave reactors are introduced in

*sibaprasad.bhattacharyya@fnlcr.nih.gov.

Supporting information: Comparative HPLC chromatograms (CH vs. MW) of crude product (Figure S1); HPLC chromatograms of Sep-Pak purified product (Figure S2) and QC data (Table S1) are included as supporting information.

radio-tracer synthesis as an alternative to CH.^{3,4} Many articles have been published over the last decade showing that the radiochemical yield and quality of many PET radio-tracers were improved by utilizing the microwave reactor as heating source.^{3–8} These studies have prompted us to use this technique in our tracer development laboratory to improve the yield and quality of the new and existing ¹⁸F-PET radiotracers.

¹⁸F-fluoroestradiol ([¹⁸F]-FES), a steroid-based PET tracer, has emerged as a dependable tracer for evaluating and managing estrogen receptor-positive (ER+) breast cancer patients.^{9–13} The first synthesis of [¹⁸F]FES was reported by Kiesewetter et al.¹⁴ In the past few years, several research groups have reported [¹⁸F]FES production using CH.^{9,14–16} However, most of these syntheses provided a low, unstable radiochemical yield with unwanted impurities in the product.^{13–15} To overcome these synthetic difficulties, a significant amount of work was conducted on [¹⁸F]-FES synthesis using CH, but no considerable effort has been made to use the MW technique. In 1999, Hostetler et al. attempted to improve the ¹⁸F-FES yield by microwave heating of the triethylammonium triflate precursor without any success.¹⁷

Considering the superiority of MW over CH, we sought to employ microwave reactor system to improve radiochemical yields and reduce the production time of [¹⁸F]-FES PET probe. In this study, we compare [¹⁸F]fluoride incorporation and hydrolysis using MW and CH with a commercially available precursor for [¹⁸F]FES synthesis (Fig. 1). We have also checked the quality of the product produced by MW for potential clinical use.

2. Materials and Methods

2.1. General

The precursor, 3-methoxymethyl-16 β , 17 β -epiestriol-O-cyclic sulfone (MMSE) and authentic standard [¹⁹F]FES were obtained from ABX, Germany. Solvents and reagents were purchased from Sigma (Milwaukee, WI) and were used without further purifications. Sterile vial, USP grade 0.9% NaCl, and sterile water for injection were purchased from Hospira. [¹⁸F]fluoride trapped in a QMA cartridge was purchased from Cardinal Health (Beltville, MD) or from PETNET (Philadelphia, PA). Sep-Pak C18 light cartridge was purchased from Waters Corporation. Before use, the cartridge was flushed with 10 mL of ethanol, followed by 10 mL of sterile water.

For MW, we used a microwave reactor (Biotage initiator classic). Microwave reaction vials, a cap with septum, and magnetic stirring bars were purchased from Biotage. Radio-thin layer chromatography (TLC) analysis of the reaction mixtures was performed on Merck silica gel 60 F254 aluminium-backed TLC plates. Radioactivity was counted in a gamma well counter (Capintec, Inc.). The semi preparative HPLC module of Eckert & Ziegler synthesizer equipped with a semi-prep C18 column (9.4 $\times$ 250 mm, 5 μ m, Zorbax-SB-Phenyl, Agilent), UV detector (fixed λ = 254), and a radiation detector, was used to purify the crude product at a flow rate of 3 mL/min, using 50% ethanol in injectable water as the mobile phase. Analytical HPLC-analysis for the quality control (QC) of the final tracer product was carried out on a Shimadzu HPLC system, equipped with auto injector, a variable UV detector preset to 280 nm and a radioactive detector (Carroll and Ramsey

Associates, CA). The samples were injected (10 μ L per injection) on-to an analytical C18 column (Phenomenex Gemini, 4.6 $\times$ 150 mm, 110 A, 5 μ m), which was eluted with a mobile phase of 50% ethanol: 50% water (v:v). The column flow rate is 0.5 mL / min and is kept at approximately room temperature, 25 to 30°C. CH was performed in a digital conventional heating block (Hach Company, Loveland, CO), with temperature stability $\pm$ 2 °C. Chemicals and supplies for QC were used as per the USP standards described in recent literature.⁹

2.2. [¹⁸F]-Labeling procedure

For each fluorination reaction, 15–20 mCi of [¹⁸F]fluoride was dried down under nitrogen, vacuum, and 110 °C in a sealed (cap with septum) Biotage glass vial (2–5 mL) containing Kryptofix 2.2.2 (12 mg), acetonitrile (0.6 mL), and K₂CO₃ solution (0.25 M) in water (0.3 mL). The residue was further dried by azeotropic distillation with anhydrous acetonitrile (2 $\times$ 0.6 mL). The FES precursor (MMSE, $\sim$ 1.0 mg) dissolved in $\sim$ 1.0 mL of anhydrous acetonitrile was added to the vial. To generate [¹⁸F]fluoride incorporation data, the vial was heated at various temperatures and times in either the microwave reactor or in a CH block. Silica TLC plates were spotted with the reaction mixture at the appropriate time points and run using methanol as the mobile phase. The plates were then analyzed for fluoride incorporation using the radio-TLC technique. To generate comparative [¹⁸F]fluoride incorporation data, [¹⁸F]fluorination was carried out for 20 min at 80, 90, 100, and 110 °C in a microwave reactor or in CH block.

2.3. Hydrolysis

To understand the impact of MW on hydrolysis, the MMSE precursor was first fluorinated by MW (110 °C for 4 min). The fluorinated intermediate was then hydrolyzed using 2 M HCl (1.5 mL) at 120 °C for 3 min in either the microwave reactor or CH block. The mixture was then neutralized with 2 mL of 4.2% NaHCO₃. The percentage of hydrolysis was determined by semi-prep HPLC using 50 % ethanol as the mobile phase with a flow rate of 3 mL/min.

2.4. Chemical purity and radiochemical yield

To compare chemical purity and radiochemical yield, [¹⁸F]FES was synthesized following the microwave procedure (fluorination at 110 °C for 4 min, hydrolysis at 120 °C for 3 min) and also by CH (fluorination 110 °C for 15 min; hydrolysis at 120 °C for 20 min). The crude [¹⁸F]FES product obtained from microwave or CH was neutralized with 2.0 mL of 4.2% NaHCO₃ and the mixture was injected into an analytical column (Phenomenex, Gemini, C-18) to determine the comparative radiochemical and chemical purities. The overall radiochemical yield was determined by purifying the product by HPLC.

2.5. Purifications

The neutralized crude product was purified by semi-prep HPLC on a Zorbax-SB-phenyl column using 50% ethanol with a flow rate of 3 mL/min. HPLC purification takes about 17 min. Instead of HPLC, a solid phase extraction (SPE) technique¹⁸ was used to purify the crude product obtained by microwave synthesis. Using this technique, crude product was neutralized with 2.0 mL of 4.2% NaHCO₃ and was further diluted with 5 mL of water.

Diluted product was trapped in an activated C-18 Sep-Pak light cartridge. Two more activated C-18 light Sep-Pak cartridges were connected in front of the [^{18}F]FES trapped cartridge in a series. The trapped product was then washed with ~ 5 mL of 40 % ethanol. The final pure product was eluted by passing through 1.0 mL of absolute ethanol. Purification by SPE takes about 5 min.

2.6. QC of microwave-assisted [^{18}F]FES

The microwave-produced [^{18}F]FES was eluted from semi-prep HPLC through a 0.22 μm sterilizing filter into a vented sterile vial previously filled with 15 mL of saline for injection, 10 mL sterile water for injection, and 0.75 mL sodium phosphate. Samples were then removed for analysis of product quality. In our laboratory, we have established (approved Investigational New Drug) QC test procedures for [^{18}F]FES prepared in an automated synthesizer.⁹ The same QC test procedures have been used to check the quality of [^{18}F]FES produced by MW (Table S1). In order to quantify the mass of FES drug and UV impurities per injected [^{18}F]FES dose (~ 6 mCi) a standard calibration curve (conc. vs UV peak area) was developed using FES authentic standard compound (ABX, Germany) at different concentration (0.06 to 1.0 $\mu\text{g/mL}$). UV peak area of the [^{18}F]FES drug product was fit on the calibration curve to determine the FES concentration. The amounts of UV impurities were measured using the same calibration curve assuming that molar extinction coefficient of UV impurities is same as FES at 280 nm wavelength.⁹

3. Results and Discussion

3.1. [^{18}F]-Fluoride incorporation: MW vs. CH

In the fluorination reaction, the [^{18}F] fluoride ion (nucleophile) is incorporated to the FES precursor (MMSE) through a nucleophilic substitution reaction to produce the [^{18}F]fluorinated intermediate (Fig. 2). Fig. 3 showed the [^{18}F]fluoride incorporation yields which were calculated from radio-TLC data (averages and standard deviations are shown in Table 1).

Initial experiments showed the [^{18}F]fluoride incorporation yields were almost unchanged after 20 min. For this reason, [^{18}F]fluorination reactions were only monitored for 20 min for both MW and CH. For all temperatures examined, the [^{18}F]fluoride incorporation was enhanced using MW as compared to CH.

Because [^{18}F]fluoride is a short lived ($t_{1/2} = 109$ min) isotope, achieving rapid and high [^{18}F]fluoride incorporation are the primary goals in radiosynthesis of ^{18}F -labeled radiopharmaceuticals. It was observed that > 90% (Fig. 3A) [^{18}F]fluorination can be achieved within 4 min in a microwave reactor at 110 $^{\circ}\text{C}$, whereas at same temperature, CH needed > 20 min to reach that level of [^{18}F]fluoride incorporation (Fig. 3B). Fig. 4 showed the comparative (MW vs. CH) [^{18}F]-fluoride incorporation rate, with temperatures at 4 and 8 min reaction times. At each temperature, MW-assisted [^{18}F]-fluoride incorporation was 30–40% higher than CH-assisted [^{18}F]-fluoride incorporation.

3.2. Hydrolysis: MW vs. CH

Hydrolysis of the [^{18}F]fluorinated FES intermediate is usually performed by CH in acid medium at high temperature to eliminate both methoxymethyl (MOM) and the sulfuryl group (Fig. 5). Based on the literature, effective hydrolysis of the intermediate is challenging.^{9,15,19} It often generates unwanted side products and/or a partially hydrolyzed polar intermediate. For [^{18}F]FES synthesis, we used an automated synthesizer (Eckert and Ziegler)⁹ where we had to repeat the hydrolysis reaction twice by heating the reaction mixture with 0.6 mL of 2N HCL at 120 °C for 10 min each time. Therefore, total hydrolysis time was about 20 min to achieve optimum yield (10–20% uncorrected, $n = 25$) with an acceptable level of UV impurities. Most of the time, we observed 5–10 % of radiolabeled side product at 6–9 min (t_R) during semi-prep HPLC, which we believe to be the [^{18}F]fluorinated intermediate. To optimize [^{18}F]FES synthesis in GE TracerLab MX automated synthesizer, Oh et al. had to repeat hydrolysis process three times.¹⁹

From the comparative [^{18}F]fluoride incorporation study it is concluded that the quickest and highest [^{18}F]fluorination can be achieved by MW at 110 °C for 4 min. In order to investigate the advantage of MW over CH on hydrolysis of the [^{18}F]fluorinated intermediate, the MMSE precursor was first fluorinated at 110 °C for 4 min by MW to achieve maximum [^{18}F]fluorine incorporation. MW hydrolysis of this fluorinated intermediate using 1.5 mL of 2N HCL at 120 °C for 3 min showed > 95% conversion to the product (based on semi-prep HPLC; Fig. 6A). On the other hand, CH hydrolysis of this fluorinated intermediate at 120 °C for 3 min showed only about 5% conversion to the final product. Most of the radioactivity remained as fluorinated intermediate ($t_R = 7$ –9 min) as shown in Fig. 6B. We achieved around 80% of hydrolysis yield by conventionally heating the intermediate with 1.5 mL of 2N HCL for 20 min at 120 °C (Fig. 6C). In semi-prep (Fig 6) earlier broad radiation peak is due to the close vicinity of radiation detector to the HPLC column (E & Z HPLC module).⁹ Radiation detector is sensing radiation while compound is moving inside the column. This broad peak was collected separately and the radioactivity associated with this peak was measured and found to be negligible (few μCi).⁹ Analytical HPLC didn't show any noticeable UV peak. Collected major product peak was extremely pure and suitable for medical use.

3.3. Radiochemical yield and chemical purity

As expected, enhanced and quick [^{18}F]fluoride incorporation and rapid hydrolysis using MW translated to an increase in the overall radiochemical yield of [^{18}F]FES. Isolated yield (uncorrected, after purification) using MW was significantly increased from 30% to 55% (~ 90% improvement). Reproducibility of the radiochemical yield is an important aspect of the radiopharmaceutical product. The coefficient of variation (CV; Table 2) values showed that radiochemical yields from CH are more variable than those from MW ($\text{CV}_{\text{CH}} = 23\%$, $\text{CV}_{\text{MW}} = 9.5\%$). This evidence was further supported by the observation of large fluctuations in [^{18}F]fluoride incorporation using CH compared to MW (Table 1).

The radiochemical purity of the crude product obtained by either MW or CH was comparable, whereas the chemical purity (HPLC UV trace, Fig. S1) of the crude reaction mixture was much higher for the MW reaction than for the CH reaction. HPLC radioactivity

chromatograms for both conventional and microwave reaction contained [^{18}F]FES as a major peak at ~ 10 min whereas the UV chromatogram of the CH reaction contained more UV impurities as compared to MW reaction (Fig. S1). The decreased formation of non-radioactive (UV) impurities with MW, compared to CH, may be the result of shorter reaction times and differences in the mechanism of heating.²

Because of the cleaner reaction, [^{18}F]FES obtained by MW was purified using the SPE technique by connecting three C-18 light Sep-Pak cartridges in a series. It was observed that SPE purification (5 min) was much quicker than HPLC purification (17 min) and the overall radiochemical yield of the pure product (Fig. S2) was increased by 5–10% ($n = 3$) compared to the HPLC purification yield. The decrease in radiochemical yield resulting from HPLC purification, as compared to SPE purification, may be attributed to a longer purification time and normal loss of product during semi-prep HPLC injection and product peak collection.

3.4. Rapid synthesis

[^{18}F]FES can be synthesized rapidly in one-pot with high radiochemical yield and purity using MW. [^{18}F]fluoride was dried in a sealed microwave vial. The vial was microwaved for 4 min at 110 °C after adding 1.0 mL of ACN solution of the MMSE precursor (~ 1.0 mg). The same vial was microwaved again for 3 min at 120 °C after adding 1.5 mL of 2N HCl. The reaction mixture was diluted with water and purified using either SPE or HPLC. Overall, total MW-assisted synthesis took about 38 min ($n = 3$) and included [^{18}F]fluoride drying, [^{18}F]fluoride incorporation and HPLC purification. The SPE purification technique can reduce the overall synthesis time to 30 min. Manual synthesis of [^{18}F]FES in a CH system takes about 70 min, and radiochemical yield is much lower compared to the yield produced using the MW technique. Automated synthesis in commercially available synthesizers (Eckert & Ziegler, GE TracerLab MX, ASU-Comecer, etc.) systems equipped with a CH reactor takes about 80 min, with 10–20% uncorrected radiochemical yield.^{9,16,19}

3.5. Quality control for medical use

[^{18}F]FES is in clinical trial as a potential PET imaging probe for ER+ breast cancer patients. Many trial sites including ours do not require more than two to three patient doses per day. Typical dose amount injected per patient is ~ 6 mCi.⁹ In routine clinical production, using an automated synthesizer, we typically get 20–40 mCi ($n > 25$) of injectable [^{18}F]FES product, starting from ~ 200 mCi of F-18 activity. Using the microwave technique, starting from ~ 100 mCi of activity, we had reproducibly isolated (HPLC purified) 50–60 mCi ($n = 3$) of [^{18}F]FES. We performed all the QC tests on two HPLC purified batches of the microwave-assisted [^{18}F]FES product, following USP procedures for clinical-grade PET imaging drugs.⁹ Results showed that [^{18}F]FES obtained by MW met all criteria including chemical and radiochemical purity (Figure 7) for medical use (Table S1). Starting with a small amount of activity (~100 mCi), more than five patient doses can be dispensed quickly. Production of [^{18}F]FES using MW will not only reduce the production time, but also improve the yield and product quality.

4. Conclusion

A comprehensive comparison of microwave-assisted heating and CH has been performed for the [^{18}F]fluorination and hydrolysis of the commercially available [^{18}F]FES precursor (MMSE). MW resulted in increased [^{18}F]fluoride incorporation and quick one step hydrolysis (3 min) to produce clinical quality [^{18}F]FES with improved isolated radiochemical yield (55 % from 30%, ~ 90% improvement). Microwaving further displayed clear advantages over CH by allowing for significant with reductions in reaction and purification (SPE vs. HPLC) times, while maintaining reproducible high radiochemical yield and quality for clinical use. Therefore, microwave-assisted radiosynthesis should be a preferred (quicker, safer and cost-effective) method for producing [^{18}F]FES. This work further indicates that an automated synthesizer equipped with a microwave reactor would be the ideal equipment for the efficient routine synthesis of [^{18}F]-FES for both clinical and preclinical research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This project has been funded in whole or in part with federal funds from the A, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U. S. Government.

References

1. Mingos DMP, Baghurst DR. Application of microwave dielectric heating effects to synthetic problems in chemistry. *Chem. Soc. Rev.* 1991; 20:1–47.
2. Belanger AP, Pandey MK, DeGrado TR. Microwave-assisted radiosynthesis of [^{18}F]fluorinated fatty acid analogs. *Nucl. Med. Biol.* 2011; 38(3):435–441. [PubMed: 21492792]
3. Elander N, Jones JR, Lu SY, Stone-Elander S. Microwave-enhanced radiochemistry. *Chem. Soc. Rev.* 2000; 29:239–249.
4. Stone-Elander, S.; Elander, N.; Thorell, JO.; Fredriksson, A. *PET Chemistry*. Vol. Chapter 9. Springer: 2007. Microwave in F-18 chemistry: quirks and tweaks; p. 244-266.
5. Miller PW, Long NJ, Vilar R, Gee AD. Synthesis of ^{11}C , ^{18}F , ^{15}O and ^{13}N radiolabels for positron emission tomography. *Angew. Chem. Int. Ed.* 2008; 47:8998–9033.
6. Lu S-Y, Chin FT, McCarron JA, Pike VW. Efficient O- and N-(β - fluoroethylation)s with NCA [^{18}F] β -fluoroethyl tosylate under microwave enhanced conditions. *J Labelled Cpd. Radiopharm.* 2005; 47:289–297.
7. Riss PJ, Roesch F. Efficient microwave-assisted direct radiosynthesis of [^{18}F]PR04.MZ and [^{18}F]LBT999: selective dopamine transporter ligands for quantitative molecular imaging by means of PET. *Bioorg. Med. Chem.* 2009; 17:7630–7634. [PubMed: 19846314]
8. Mandap KS, Ido T, Kiyono Y, Kobayashi M, Lohith TG, Mori T, et al. Development of microwave-based automated nucleophilic [^{18}F]fluorination system and its application to the production of [^{18}F]flumazenil. *Nucl. Med. Biol.* 2009; 36:403–409. [PubMed: 19423008]
9. Dixit M, Shi J, Wei L, Afari G, Bhattacharyya S. Synthesis of clinical-grade [^{18}F]fluoroestradiol as a surrogate PET biomarker for the evaluation of ER-targeting therapeutic drug. *Int. J. Mol. Imaging.* 2013; 2013:1–10.

10. Peterson LM, Mankoff DA, Lawton T, et al. Quantitative imaging of estrogen receptor expression in breast cancer with PET and ^{18}F -fluoroestradiol. *J. Nucl. Med.* 2008; 49:367–374. [PubMed: 18287268]
11. Dehdashti F, Mortimer JE, Siegel BA, et al. Positron tomographic assessment of estrogen receptors in breast cancer: comparison with FDG-PET and in-vitro receptor assays. *J. Nucl. Med.* 1995; 36:1766–1774. [PubMed: 7562040]
12. Kumar R. Targeted functional imaging in breast cancer. *Eur. J. Nucl. Med. Mol. Imaging.* 2007; 34:346–353. [PubMed: 17404770]
13. Dehdashti F, Flanagan FL, Mortimer JE, et al. Positron emission tomography assessment of “metabolic flare” to predict the response of metastatic breast cancer to antiestrogen therapy. *Eur. J. Nucl. Med. Mol. Imaging.* 1999; 26:51–56.
14. Kieswetter DO, Kilbourne MR, Landvatter SW, et al. Preparation of four fluorine-18-labeled estrogens and their selective uptakes in target tissues of immature rats. *J. Nucl. Med.* 1984; 25:1212–1221. [PubMed: 6092569]
15. Lim JL, Zheng L, Berridge MS, et al. The use of 3-methoxy methyl-16 beta, 17-beta-epiestriol-O-cyclic sulfone as the precursor in synthesis of F-18 16 alpha-fluoroestradiol. *Nucl. Med. Biol.* 1996; 23:911–915. [PubMed: 8971859]
16. Kumar P, Mercer J, Doerkson C, et al. Clinical production, stability studies and PET imaging with 16-alpha- ^{18}F fluoroestradiol (^{18}F FES) in ER positive breast cancer patients. *J. Pharm. Pharm. Science.* 2007; 10(2):256S–265S.
17. Hostetler ED, Jonson SD, Welch MJ, Katzenellenbogen JA. Synthesis of 2- ^{18}F fluoroestradiol, a Potential Diagnostic Imaging Agent for Breast Cancer: Strategies to Achieve Nucleophilic Substitution of an Electron-Rich Aromatic Ring with $^{18}\text{F}\text{F}^-$. *J. Org. Chem.* 1999; 64:178–185. [PubMed: 11674101]
18. Knott KE, Gratz D, Hubner S, et al. Simplified and automatic one-pot synthesis of 16 α - ^{18}F fluoroestradiol without high-performance liquid chromatography purification. *J. Labelled Comp. Radiopharm.* 2011; 54:749–753.
19. Oh SJ, Chi DY, Mosdzianowski C, Kil HS, Ryu JS, Moon DH. The automatic production of 16 α - ^{18}F FES using a conventional ^{18}F FDG module with a disposable cassette system. *Appl. Rad. Isotope.* 2007; 65:676–681.

Figure 1.
Schematic diagram of $[^{18}\text{F}]\text{FES}$ synthesis (nucleophilic fluoride incorporation followed by acid hydrolysis).

Figure 2.

Nucleophilic [^{18}F]fluoride incorporation in MMSE to form fluorinated intermediate, **a**.

K2.2.2/ K^{18}F , MW or CH.

Figure 3. ^{18}F fluoride incorporation (based on radio-TLC) in MMSE using MW (A) and CH (B) at different temperature and time.

Figure 4.
Rate of [^{18}F]fluoride incorporation at different MW or CH temperature.

Figure 5.

Acid hydrolysis of the $[^{18}\text{F}]$ fluorinated intermediate, **b**. 2N HCl, MW or CH.

Figure 6.

Semi-prep HPLC chromatograms of $[^{18}\text{F}]$ FES (Agilent Zorbax-SB-phenyl column, 50% ethanol, 3mL/min) collected in Eckert and Ziegler semi-prep HPLC module. **A.** $[^{18}\text{F}]$ fluorination: MW, 110 °C, 4 min; hydrolysis: MW, 120 °C, 3 min. **B.** $[^{18}\text{F}]$ fluorination: MW, 110 °C, 4 min; hydrolysis: CH, 120 °C, 3 min. **C.** $[^{18}\text{F}]$ fluorination: CH, 110 °C, 15 min; hydrolysis: CH, 120 °C, 20 min. Arrow in each chromatogram points to the radiolabeled intermediate. Free unreacted $[^{18}\text{F}]$ fluoride comes around 3–5 min. The two vertical solid lines in the trace show the start and end of the product collection.

Figure 7.

Analytical HPLC chromatogram of clinical grade [^{18}F] FES (HPLC purified and formulated) obtained by MW. **A)** UV and radio chromatogram of final product. Amount of UV impurities are well below the acceptable amount for human injection (5 μg per 6 mCi dose). Large UV peak is due to the presence of UV impurities in mobile phase and in formulation buffer. **B.** Authentic standard FES compound (10 μL injection of $\sim 6\mu\text{g/mL}$ of FES).

Table 1

[¹⁸F]Fluoride incorporation (%) into the precursor (MMSE) of [¹⁸F]fluoroestradiol based on radio-TLC.

Temp (°C)	Time (min)	MW	CH
80	2	30.2±2.2	15.0±2.2
80	4	41.5±2.4	17.3±4.1
80	8	59.8±3.1	30.4±3.8
80	15	61.4±1.8	39.8±4.5
80	20	65.0±2.0	52.2±3.1
90	2	63.3±2.2	26.0±3.2
90	4	77.0±3.3	48.3±7.5
90	8	83.5±4.1	59.0±5.4
90	15	91.1±3.1	69.2±8.6
90	20	93.4±2.2	78.4±5.1
100	2	80.0±3.7	25.0±6.0
100	4	88.2±3.6	55.9±4.9
100	8	89.0±4.8	65.3±5.5
100	15	91.4±3.4	78.2±4.8
100	20	92.2±2.9	83.5±6.4
110	2	83.9±3.1	28.4±7.2
110	4	92.2±2.2	59.0±4.6
110	8	93.0±2.3	73.3±7.7
110	15	92.3±3.2	83.0±6.8
110	20	92.1±2.6	87.7±7.0

n=3 at each condition

Table 2

[¹⁸F]FES isolated radiochemical yield (RCY) using MW and CH

Heating method	Purification	Isolated uncorrected RCY (%)	Coefficient of variation (%)
MW ^a	HPLC	55.0±5.2	9.5
MW ^a	Sep-pak	62.5±6.4	10.2
CH ^b	HPLC	30.5±7.1	23.0

n=3 at each condition;

^a [¹⁸F]Fluorination: MW, 110 °C, 4 min, and hydrolysis: MW, 120 °C, 3 min;

^b [¹⁸F]Fluorination: CH, 110 °C, 15 min, and hydrolysis: CH, 120 °C, 20 min.