Clinical production, stability studies and PET imaging with 16-α-[^{18}F]fluoroestradiol ([^{18}F]FES) in ER positive breast cancer patients

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ABSTRACT

Purpose: ^{18}F-Fluoroestradiol [^{18}F]FES has emerged as a valuable PET tracer to predict the response to hormone therapy in recurrent or metastatic breast cancer patients. A clinically acceptable product requires a rapid reliable synthesis and must be demonstrated to maintain chemical stability and receptor specific uptake during patient studies. [^{18}F]FES then becomes a dependable tracer for the evaluation and management of breast cancer patients. **Methods:** An improved automated radiosynthesis of [^{18}F]FES was developed. Stability studies of the injectible form of [^{18}F]FES were performed up to 24 h after dose formulation under normal storage conditions. A comparative FES/FDG PET imaging in ER+ breast cancer patients is reported. **Results:** The improved synthesis procedure utilizes fewer hydrolysis steps and a single high performance liquid column chromatography (HPLC) purification of the labeled mixture affording [^{18}F]FES in good yield with high radiochemical purity (>99%). Stability studies with purified [^{18}F]FES in saline/ethanol (85:15 v/v) indicated no radiolytic or chemical degradation of this radiopharmaceutical when stored for 24 h at 20-24 °C. Positron Emission Tomography (PET) studies with [^{18}F]FES and [^{18}F]FDG in estrogen receptor positive (ER+) breast cancer patients indicated that while FDG accumulation was seen in all metabolically hyperactive sites, the uptake of FES clearly delineated the ER+ tissues regions. **Conclusions:** An improved automated synthesis of [^{18}F]FES has been developed and the integrity of this product has been validated by long term stability studies and clinical PET imaging studies in ER$^+$ breast cancer patients. A lack of concordance between FES and FDG uptake in a patient with metastatic breast cancer suggests specificity of the FES for tumors expressing estrogen receptors.

INTRODUCTION

The future evolution of positron emission tomography (PET) imaging is dependent upon the development and introduction into clinical and translational practice of new targeted radiotracers that can serve as predictive assays of treatment response, in-vivo biomarkers that will permit stratification to the most appropriate therapy and individualization of patient treatment plans, and which can identify failure of therapy patients on an individual basis (1). Imaging with [^{18}F]FDG, whilst highly effective as a diagnostic or staging radiopharmaceutical, has limitations in acting as an imaging biomarker as defined above (2).

[^{18}F]FES is a steroid based PET radiotracer that can be used to determine, in-vivo, the tissue levels of the estrogen receptor in patients with breast cancer and may emerge as a valuable tool to help predict which patients with primary, recurrent or metastatic breast cancer will respond to hormone therapy (3-5). If this potential can be validated, then [^{18}F]FES may evolve as one of a new generation of PET radiotracers that can be used with confidence as an imaging biomarker.

Improved automated procedures are designed not only to minimize the radiation exposure to laboratory personnel but also to enhance the efficiency and quality of reactions by reducing the production time. The consistency that can be achieved by automated syntheses also produces a radiopharmaceutical product that will be more acceptable to regulatory agencies. The automated procedure described in the literature for [^{18}F]FES is particularly complex due to the requirement for high performance liquid chromatography (HPLC) purification of both an intermediate in the synthesis and the final product (9).

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In addition three separate hydrolysis steps are required to ensure complete removal of blocking groups to produce the final crude product (10-12). The stability of [^{18}F]FES in an injectable formulation under normal storage may be an issue for its clinical utility particularly since a recent study reported that the radiochemical quality of [^{18}F]FES deteriorated rapidly when it was stored in saline (13).

This prompted us to develop an optimized automated procedure to synthesize [^{18}F]FES using an in-house modification of a commercial ASU and validate the chemical and radiochemical integrity of this product through long term stability studies (up to 24 hours), extensive quality control tests and its estrogen receptor specific expression in breast cancer patients. The synthesis refinements include a reduced number of hydrolysis steps and product isolation with a single terminal purification step using HPLC. These modifications not only reduced the total time of synthesis and purification but also afforded [^{18}F]FES in appropriate yields for clinical studies. To date 20 joint clinical imaging studies with [^{18}F]FES/ ^{18}F-fluorodeoxyglucose [^{18}F]FDG have been carried out in patients at our centre that confirm the utility of [^{18}F]FES as an estrogen receptor imaging biomarker in conjunction with [^{18}F]FDG acting as a metabolic marker of breast cancer. The goal of the study is to establish the radiochemical and clinical comparability of our product with that reported in the literature (13-15).

EXPERIMENTAL SECTION

Materials and methods

Synthesis of [^{18}F]FES was performed in an ASU purchased from Advanced Cyclotron Systems (Richmond, British Columbia, Canada) and housed in a commercial radioisotope isolator unit designed to confer a Class 100 operating environment during ASU operation (Comecer, Milan, Italy). Automatic functions of this unit, such as time controlled selective valve openings and closings, heating and cooling, are operated through a control software interface (Lookout for Win32 Version 4.5.1, National Instruments, USA). A schematic of the physical components of the ASU is shown in Fig. 1.

FES precursor, Compound **1** (Scheme 1), and the reagents for use in the automated synthesis were manufactured and sterile packed by the Edmonton Radiopharmaceutical Centre (Edmonton, Alberta, Canada). Authentic reference FES was obtained from ABX Biochemicals (Radeberg, Germany). ^{18}F-fluorine was produced at the Edmonton PET Centre using an Advanced Cyclotron System TR 19/9 cyclotron with approximately 17.5 MeV protons at 20 μA on a highly enriched $H_2{}^{18}O$ water target making use of the $^{18}O(p,n)^{18}F$ nuclear reaction. The reaction vial (10 mL) was supplied by Ace, USA. A Beckmann High Performance Liquid Chromatography (HPLC) system was used to purify and perform the quality assurance checks on the synthesized ^{18}F-FES. HPLC purification was carried out on a reverse phase 10-ODS-3 column 25 x 0.9 cm (Whatman Inc. New Jersey, USA,) and the labeled product was identified using dual detectors (UV at 290 nm and radioactivity detection).

Radiochemistry

Automated synthesis of ^{18}F-FES was performed according to Scheme 1. The reaction sequence for the ASU was preceded by a thorough cleaning of the transfer lines and a drying procedure followed by the attachment of the reagent vials at designated positions on the ASU that were sampled to provide the necessary components for the synthesis. The reagents used in this synthesis are as below.

a) Eluent vial containing a solution of Kryptofix®-222 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane; 22 mg) and potassium carbonate (7 mg) in 0.8 mL of sterile water:acetonitrile (50:50 v/v).
b) 3-O-Methoxymethyl-16,17-O-sulfuryl-16-epiestriol, the sulfone precursor 1 (1.0-1.2 mg, 2.5-3.0 μmol) dissolved in 2 mL anhydrous acetonitrile.
c) Anhydrous acetonitrile, 2 mL
d) HCl (0.1N in acetonitrile, 1.5 mL)
e) Ethanol:water 1.5 mL (1:1 v/v)

Reagents were transferred to the reaction vessel in appropriate volumes through the application of vacuum or dry nitrogen gas pressure and automated timed opening and closing of appropriate valves. All reactions occurred within the reaction vessel which could be rapidly heated and cooled as required. All radioactivity handling steps were

performed while the ASU was contained within the shielded Class 100 environment. Radiation monitors allowed the transfer and progress of the radioactive components to be observed.

In the optimized reactions no-carrier-added (NCA) radiofluoride ($^{18}F^-$) was first adsorbed from the irradiated $H_2{}^{18}O$ target solution onto an activated anion exchange resin cartridge (Waters, QMA).

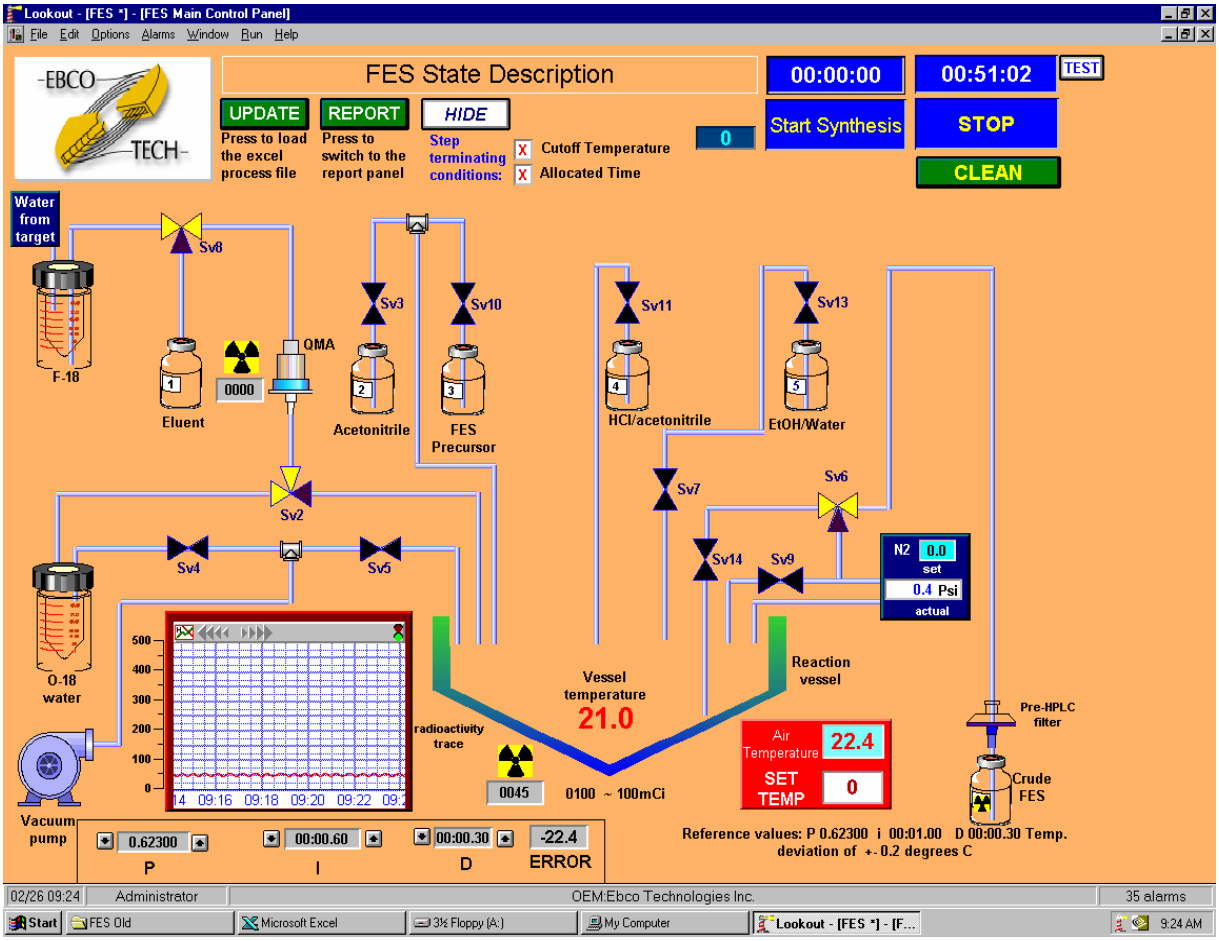
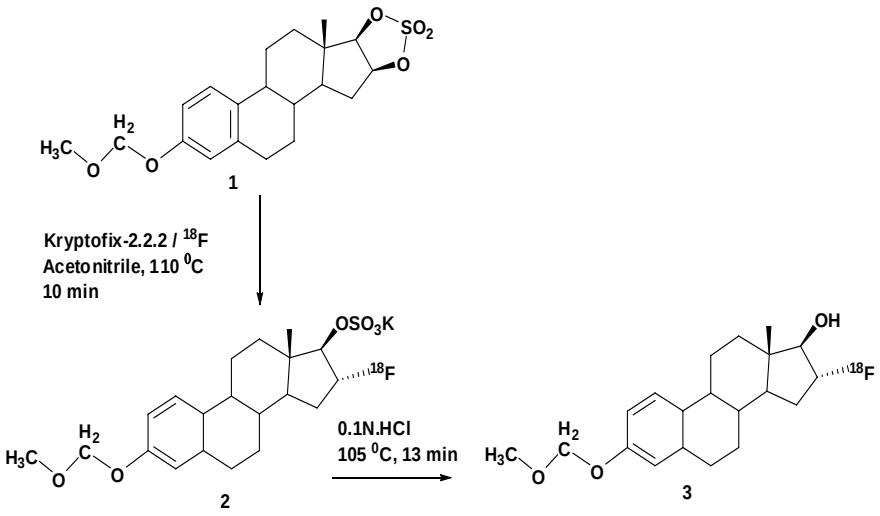


Figure 1. Schematic layout for the Advanced Technologies System Automatic Synthesis Unit



Scheme 1. Radiosynthesis of ^{18}F-Fluoroestradiol ([^{18}F]FES).

Table 1. Major Automated Synthesis Unit reaction control parameters

Parameter	Reaction step	Set point value	Set-point control
Activated radiofluoride drying time	Activated radiofluoride in AcCN solution to anhydrous solid activated radiofluoride	18 min	± 1.0 s
Activated radiofluoride drying temperature (vacuum)	Activated radiofluoride in AcCN solution to anhydrous solid activated radiofluoride	95 °C (80 kPa)	± 0.1 °C (±5 kPa)
Fluorination reaction time	Dry activated radiofluoride and MOM estratriol-sulfone to produce MOM [^{18}F]FES sulphate	10 min	± 1.0 s
Fluorination reaction temperature	Dry activated radiofluoride and MOM estratriol-sulfone to produce MOM ^{18}F-FES sulphate	110 °C	± 0.1 °C
MOM [^{18}F]FES sulphate hydrolysis reaction time	MOM ^{18}F-FES sulphate hydrolysis to [^{18}F]FES	13 min	± 1.0 s
MOM [^{18}F]FES sulphate hydrolysis reaction temperature	MOM ^{18}F-FES sulphate hydrolysis to [^{18}F]FES	105 °C	± 0.1 °C

Table 2. Typical amounts of [^{18}F]fluoride and percent yields of [^{18}F]FES

Experiment	^{18}F activity (GBq)			Decay corrected yield (%) ([^{18}F]FES x 100 / [^{18}F]F$^-$)
	Start of synthesis as ^{18}F$^-$	End of synthesis as crude [^{18}F]FES	After purification as [^{18}F]FES	
1	17.8	8.71	1.59	18.9
2	14.6	7.81	1.35	17.5
3	33.7	12.66	5.06	26.8
4	34.4	10.43	4.04	22.5
5	26.2	9.43	3.79	26.1
Average Yield ± Standard Deviation				22.4 ± 3.3

Cartridges were activated by the sequential passage of potassium carbonate (0.5M, 10 mL) sterile water, (10 mL) and nitrogen gas (to dryness). The trapped ^{18}F was transferred to the reaction vial by passage of the contents of the elution vial. After two azeotropic evaporations of the solvent, using repeated additions of anhydrous acetonitrile at preset heat (95 °C) and vacuum (80 kPa) with nitrogen carrier gas flow (34 – 55 kPa), the residual traces of water were completely removed. The activated ^{18}F, on reaction with sulfone precursor **1** in anhydrous acetonitrile at 110 °C, afforded the methoxymethyl (MOM) blocked [^{18}F]FES sulfate **2**. Evaporative acid hydrolysis of this protected intermediate was carried out using two separate additions of 0.1N HCl in acetonitrile (0.75 mL per addition) at 105 °C under nitrogen flow with applied vacuum. The crude [^{18}F]FES, **3**, so obtained, was dissolved in 1.5 mL of 50% aqueous ethanol and then dispensed from the ASU through a sterile Millex HV filter (0.22 μm) into a shielded sterile product vial. The major ASU reaction control parameters are given in Table 1.

HPLC purification

[^{18}F]FES solution, as dispensed from the ASU, was injected onto an HPLC system equipped with a 5 mL injection loop, guard column, reversed phase C-18 column, UV detector, radioactivity detector, and was eluted with ethanol-water (52:48; v/v) at a flow rate of 1.8 mL/min (preparative conditions). ^{18}F-FES, under these HPLC conditions, appeared at a retention time of 13.5 (±5%) min. The terminal end of the elution line was connected to a sterilized manifold, containing an alumina cartridge and a 0.22 μm Millex LG filter. Purified [^{18}F]FES, after elution, passed through this sterilized set up and was collected in to a pre-weighed sterile multi-dose vial (20 mL) that contained (10 mL) sterile saline. The results indicating absolute isolated yields of [^{18}F]FES are given in Table 2.

Quality control of [^{18}F]FES

Chemical and radiochemical purity of ^{18}F-FES was confirmed on a reverse phase HPLC column (25 x 0.9 cm, 10 ODS 3) using ethanol/water (40/60; v/v) as eluent at a flow rate of 1.8 mL/min (analysis conditions). A major radioactive peak was detected corresponding to >98% of the eluted radioactivity at a retention time of 17.5 (±5%) min. This elution time corresponded to [^{18}F]FES as confirmed by co-elution with authentic non-radioactive FES. Additional quality control checks included the determination of residual Kryptofix®-222, radionuclidic half life (105-115 min), product clarity, pH (6-7) and a retrospective testing for sterility and apyrogenicity.

Stability

Detailed stability studies were performed to confirm the suitability of the final product for clinical use. Appearance, pH, radiochemical identity and radiochemical purity were included in this study as reflected in Table 3. Factors that might affect stability included chemical and radiolytic decomposition, temperature and vial orientation.

Three batches of purified [^{18}F]FES containing high activity (> 2 GBq) were included in this study. Each batch of [^{18}F]FES, collected as 15% EtOH solution in saline directly from the HPLC purification without dilution, was divided into two separate vials. One vial was stored upright and the other one in inverted position for the stability test period.

Temperature was maintained between 20-24 °C. Samples from each vial were withdrawn at a time period of 0, 4, 8 and 24 hours and checked for changes in appearance and pH. Radiochemical purity and identity of [^{18}F]FES were confirmed by co-injecting the test samples at defined time periods with standard FES on an HPLC chromatography system and analyzing the uv and radioactive peak areas which eluted along with standard FES. The results of this study are presented in Table 3. The 2 hour half-life of ^{18}F precluded the determination of product purity and identity at 24 hours.

Table 3. Stability Lot data for [^{18}F]FES in high activity formulation*

QC Test	Elapsed Time (h)	Lot Number FES 1504		Lot Number FES 1704		Lot Number FES 1804	
		Upright	Inverted	Upright	Inverted	Upright	Inverted
Appearance	0	Clear	Clear	Clear	Clear	Clear	Clear
	4	Clear	Clear	Clear	Clear	Clear	Clear
	8	Clear	Clear	Clear	Clear	Clear	Clear
	24	Clear	Clear	Clear	Clear	Clear	Clear
pH	0	6.5	6.5	6.5	6.5	6.5	6.5
	4	6.5	6.5	6.5	6.5	6.5	6.5
	8	6.5	6.5	6.5	6.5	6.5	6.5
	24	6.5	6.5	6.5	6.5	6.5	6.5
Radiochemical Identity (RT)**	0	N/D	N/D	13.56	13.56	14.15	14.15
	4	13.71	13.36	13.80	13.42	13.68	N/D
	8	13.64	13.71	13.63	13.71	13.63	13.60
	24	Decayed	Decayed	Decayed	Decayed	Decayed	14.0
Radiochemical Purity	0	100	100	100	100	98.8	98.8
	4	100	99.9	100	100	98.9	99.9
	8	100	100	100	100	100	100
	24	Decayed	Decayed	Decayed	Decayed	Decayed	100

*Activity > 2GBq.
**Radiochemical identity of test samples was confirmed using HPLC by comparing the retention time of [^{18}F]FES which is +/- 5% of the retention time for standard FES
(N/D = not done)

PET IMAGING

Imaging protocol

To validate the [^{18}F]FES production, we established a Phase II imaging protocol to compare images obtained with [^{18}F]FES with those obtained with [^{18}F]FDG. Twenty patients with metastatic breast cancer have been imaged to date. Local research ethics board approval was obtained for the Phase II protocol, and written informed consent for each of the two imaging studies was obtained from each patient. Imaging with [^{18}F]FDG was performed using published guidelines (16) on a Philips Allegro PET scanner (Cleveland, Ohio, USA). One day after the [^{18}F]FDG imaging, patients were injected with [^{18}F]FES (typical dose 300 – 400 MBq). Images were obtained one hour after injection; 5 – 7 bed positions were acquired, for a total of 5,000,000 counts using the same scanner as the FDG scan. [^{18}F]FES was prepared on demand in lots for one or two patients and imaging was initiated within 2 hours of the end of synthesis for the first patient and within 3 hours for the second patient. Data were also recorded to identify adverse reactions to the administration of the [^{18}F]FES.

Image review

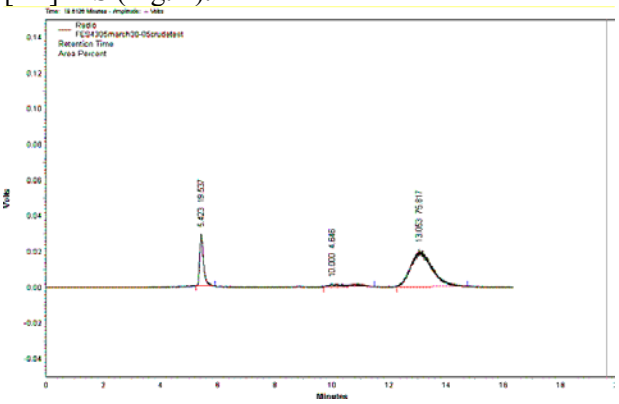
[^{18}F]FES and [^{18}F]FDG images were reviewed by two experienced nuclear medicine physicians, and compared for sites of abnormal uptake. In addition the biodistribution of [^{18}F]FES produced by the method described in this manuscript was compared with that reported in the literature (17).

RESULTS

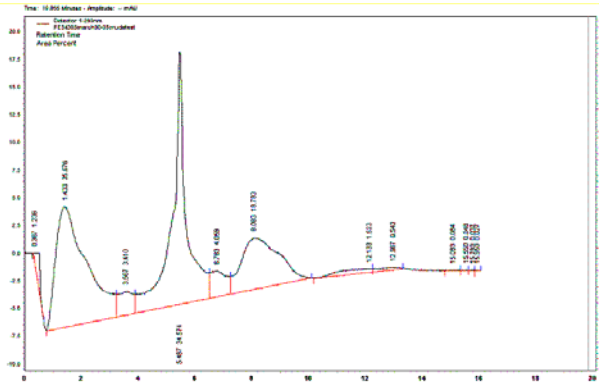
Radiochemistry

The synthesis of [^{18}F]FES was achieved as shown in Scheme 1 by reacting the sulfone precursor **1** (1-1.2 mg) in anhydrous acetonitrile with dry ^{18}F/K-2.2.2 complex at 110 °C for 10 min followed by acid catalyzed removal of the methoxymethyl and sulfate protective groups. Evaporative hydrolysis using 0.1N HCl acid in acetonitrile (0.75 mL x 2) at 105 °C afforded impure ^{18}F-FES which was dispensed from the ASU as an aqueous ethanol solution (1.5 mL; 50% ethanol in sterile water). Total synthesis time from the start of the fluoride elution from the cartridge to the isolation of the final crude product ready for HPLC purification was 58 minutes. The timings for various steps in the reaction sequence are detailed in Table 1.

The crude [^{18}F]FES in 1.5 mL of 50% aqueous ethanol was purified using a single HPLC run requiring an additional 15 minutes. HPLC analysis of the unpurified solution (crude [^{18}F]FES) indicated that the mixture contained 70-85% of [^{18}F]FES (Fig. 2).



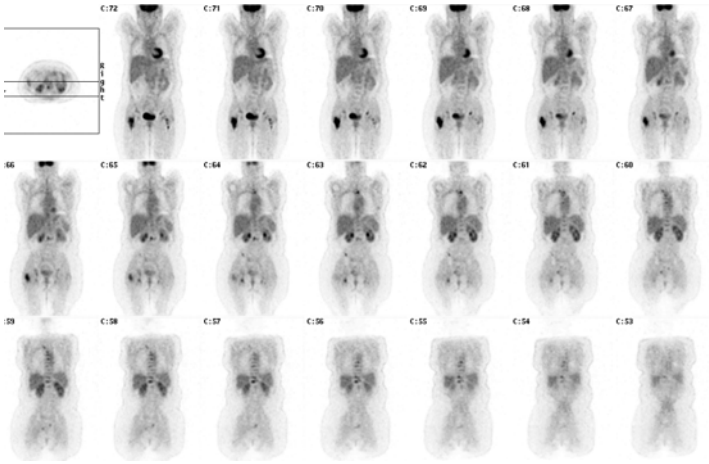
2a



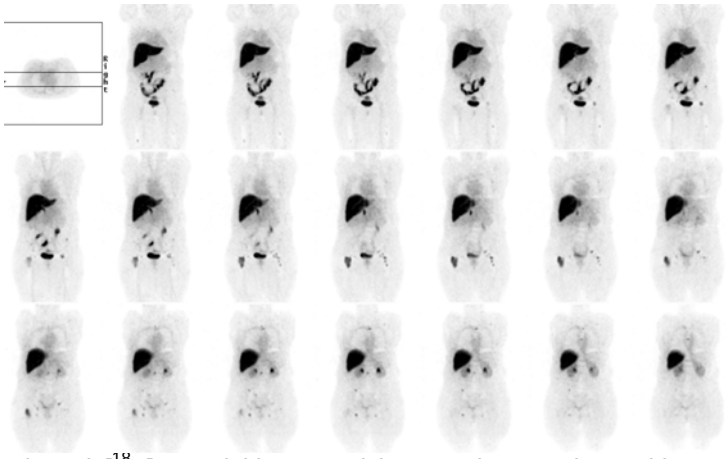
2b

Figure 2. HPLC analysis of the [^{18}F]FES reaction mixture as seen on radioactivity (2a) and UV (2b) channels; Retention times: 5.4 min = unreacted fluoride; 13.1 min = [^{18}F]FES (Fig 2a) and 7.7-9.8 min = epiestriol (2b).

For clinical studies the HPLC purified ^{18}F-FES was collected after passage through a sterile membrane filter into a vial containing saline and was found to be at >99% radiochemical purity. The discrepancy between crude and purified yield results from the relatively broad product peak on HPLC and the collection of the central portion of this peak to provide pure clinical product free from radiochemical and chemical impurities. Through this procedure, we obtained excellent radiochemical purity at the expense of radiochemical yield. Purified [^{18}F]FES solution was further diluted in saline prior to patient injection to achieve a final ethanol concentration of <3%. Final decay corrected purified radiochemical yields in the large scale clinical preparation ranged from 15-35%.



3a



3b

Figure 3. [1[8]F]FDG (3a) and [[18]F]FES (3b) coronal images in a patient with ER +ve metastatic breast cancer. The relative differences in the biodistribution of the 2 radiotracers are demonstrated on these coronal sections which are displayed from anterior to posterior. Multiple bone metastases are identified on the [[18]F]FDG image. Note that whilst the pelvic metastases show uptake of [[18]F]FES, many of the spinal lesions do not.

Analysis of the crude reaction product indicated >75 % radiochemical yield (Fig. 2). A colorimetric tlc check of the purified preparation showed no detection of Kryptofix®-222 contaminant within specified limits (<50 μg/mL). The specific activity was interpolated from a calibration curve plotting peak area as a function of cold FES (1 μg-10 ηg).The minimum detectable amount of FES under the HPL chromatographic conditions described above was 10 ηg. Using this calibration, the specific activity of no carrier added [[18]F]FES was found in a range of 0.227-1.66 GBq/μg (1.78-12.97 Ci/μmol). None of the [18]F-FES samples showed a UV response high enough to match the minimum detectable concentration of FES.

Stability

The stability studies with purified [[18]F]FES in 15-20% ethanol solution in saline were performed up to 24 h and included pH, clarity of the solution and chemical and radiochemical purity. The radiochemical integrity of [[18]F]FES was retained between 98.9-100% under all storage conditions and times (up to 24 h in one experimental lot). No significant UV visible impurity was seen in these solutions. In addition, pH and clarity of the product solutions did not change (Table 3).

Clinical imaging

All sets of patient images showed comparable distribution of radiotracer in normal tissues at the one hour time-point. Whole body biodistribution images obtained with [[18]F]FES produced by our synthetic pathway shows its radiochemical equivalence with that reported in the literature (17). At one hour after injection the biodistribution in a patient with no ER positive disease shows the liver as the primary metabolic pathway; there is biliary excretion, and activity is seen transiting the small bowel. A small amount of renal and bladder activity is also seen, demonstrating a renal excretory pathway. No adverse reactions were recorded to any of the [[18]F]FES injections.

A patient with ER positive primary breast carcinoma with multiple bone metastases (Fig 3) is demonstrated by the [[18]F]FDG uptake images on both coronal and sagittal views (Fig 3a). The [[18]F]FES image (Fig 3b) shows specifically some ER positive bone metastases, since there is considerable discordance between the [[18]F]FES images and the [[18]F]FDG images, suggesting loss of estrogen receptors in many metastatic sites. This loss of estrogen receptor in a large number of metastatic sites is a predictive of failure to anti-estrogen therapies.

DISCUSSION

An improved procedure to synthesize [[18]F]FES is described which required a considerably smaller amount of the sulfone precursor **1** (1–1.2 mg) and with fewer hydrolysis steps than previously reported syntheses (12). HPLC purification of

impure [^{18}F]FES, dispensed from the ASU, has also been simplified by using a single purification in comparison to other reports where it was obtained by using two columns or two purifications (6, 9). Purified [^{18}F]FES is obtained directly from HPLC purification in the form of an injectable solution (<15% aqueous ethanol which is further diluted by sterile saline to lower the final ethanol content below 3% prior to the patient administration). This is an advantage over other reports where solvents had to be removed from the labeled intermediate and the purified [^{18}F]FES solution followed by reconstitution due to non-compatibility of the HPLC solvents (acetonitrile) with clinical requirements (9). The direct elution as injectable product also avoided the addition of sodium sorbate to the final [^{18}F]FES solution, used by others to minimize the decomposition (13). The [^{18}F]FES, thus prepared, was of high specific activity and high radiochemical and chemical purity.

We also confirmed the long term stability of the product and utilized it effectively in clinical studies to determine the estrogen receptor status in patients with advanced breast cancer. The stability studies with purified [^{18}F]FES in 15-20% ethanol solution in saline were designed to mimic the actual storage conditions that might be encountered during the shipping of a clinical radiopharmaceutical. The samples used for stability studies were at high radioactive concentration prior to final dilution with saline and thus provided a test environment more prone to radiolytic decomposition. The [^{18}F]FES samples showed no deterioration over 8 hours when analyzed by HPLC with additional observations confirming product integrity (pH, appearance, particulates) up to 24 hours. The radiochemical integrity of these samples after 24 h of storage could be analyzed only in one sample (Table 3, Lot FES 1804, inverted vial) due to complete decay of radioactivity in other samples although other stability parameters were found to be acceptable.

FES is under investigation by our group and a number of others as an aid in the assessment of the estrogen receptor status of metastatic and recurrent breast cancer. In this milieu it can predict the success or failure of anti-estrogen therapy as well as providing valuable information on the changes in receptor status that accompany disease progression during treatment or with disease recurrence. It is particularly useful when combined with [^{18}F]FDG imaging which will generally indicate all metabolically active lesions and not only those expressing the estrogen receptor.

This study was not intended to demonstrate either clinical efficacy, or clinical utility. Rather, it was intended to qualitatively evaluate [^{18}F]FES produced using this automated synthesis pathway by confirming its chemical and radiochemical integrity and utility in translational research by comparing its clinical estrogen receptor specific uptake with published studies in breast cancer patients (14). However, in this limited series evaluating image quality we have noted significant heterogeneity of estrogen receptors expression. This study is an example of the power of molecular imaging to stratify patients for the most appropriate therapy and confirms the potential utility of [^{18}F]FES as an imaging biomarker. If these data can be confirmed in a prospective clinical trial it is possible to envisage a role for estrogen receptor imaging in stratifying patients with primary and metastatic breast cancer for the most appropriate treatment plan. An IRB approved protocol is currently recruiting patients to test the hypothesis that imaging with [^{18}F]FES can improve patient outcomes.

CONCLUSION

Clinical preparations of PET radiotracers are most appropriately handled using automated synthesis units (ASUs) since these can provide rapid, reproducible syntheses while minimizing radiation exposure to personnel. Products produced by automation may also be more acceptable to regulatory agencies when the issues related to the radiation safety, documentation and reproducibility of clinical productions are appropriately addressed. Commercial ASUs are not available for every PET product although some manufacturers provide generic units designed for multiple potential products. It is useful, therefore, to be able to manipulate the software control and hardware of commercial units to achieve the conditions for synthesis of new products. We successfully modified an ASU from Advanced Cyclotron Systems originally designed for [^{18}F]FDG production to produce [^{18}F]FES. We were able to optimize this reaction thereby improving on existing published syntheses. The refinements included a reduction in the quantity of ligand

required in the synthesis, an improved hydrolysis of the labeled intermediate and the use of a single terminal purification by HPLC to obtain the clinical product and a consequent reduction in the overall reaction and purification time (total 73 min). We have also confirmed the long term stability of [^{18}F]FES and performed a detailed chemical and clinical validation of this product by determining the estrogen receptor status in patients with advanced breast cancer.

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