was added to the reaction vessel for fluorination and heated at 105 °C for 10 min. After the fluorination, the solution was transferred to the syringe 1 to release the increased pressure, and then returned back to the reaction vessel. The solvent was evaporated to decrease the total volume. To start the hydrolysis, D was added to the reaction vessel and heated at 95 °C for 10 min with the valves closed, which brought a pressurized condition. To stop the hydrolysis, neutralization was performed in syringe 1. The base reagent, E, was transferred to syringe 1 during the hydrolysis, and then the reaction solution was drawn in the same syringe. The mixture was pushed out to the reaction vessel to recover the remaining radioactivity and drawn again. The radioactive solution was pushed out into the crude FES collection vessel for HPLC purification. The acetonitrile in B (about 1 ml) was used to flush the reaction vessel and the cassette, and added to the crude FES vessel. The reagent F was used for flushing the cassette.

The HPLC purification and the formulation steps: The HPLC purification was performed using a Cosmosil $5C_{18}$-AR-2 column (20 mmI.D. x 250 mm, Nacalai Tesque, Kyoto, Japan) with acetonitrile/water/ethanol=30/40/30 at a flow rate of 6.0 ml/min. For the HPLC injection, we used a 5 ml sample loop and automatic injector (Rheodyne, Rohnert Park, USA). The desired radioactive fraction was collected in a round-bottom flask containing 0.1 ml of sodium ascorbate (250 mg/ml) in the solvent replacement unit (JFE P&S). The solvent was removed *in vacuo* and the residue was dissolved in 10 ml of saline, and then passed through a sterile 0.22 μm filter (Millex-GS, Millipore, MA) to a pyrogen-free vial as the final product.

Evaluation of the products

The radiochemical purity of the product right after the synthesis and in the final product for clinical use was determined by HPLC analysis using a Cosmosil $5C_{18}$-MS-2 column (4.6 mmI.D. x 150 mm, Nacalai Tesque) with 40% acetonitrile/water at a flow rate of 1.0 ml/min. In this system, [^{18}F]FES was eluted for 8.1 min. To determine the yield of fluorination, the free [^{18}F]fluoride in the crude [^{18}F]FES solution was measured by radio thin layer chromatography

(radio TLC) method. The radio TLC was performed on Merck aluminum backed silica gel 60 plates with chloroform/methanol=4/1. The Rf value of [^{18}F]fluoride and [^{18}F]FES were 0.00 and 0.75, respectively. The other two unknown labeled compounds had Rf value of 0.30 and 0.40.

RESULTS AND DISCUSSION

In this study, we established the optimal procedure for [^{18}F]FES preparation for clinical use through investigating the condition for the synthesis, the purification and the formulation steps. We chose the synthesis method using **1** as the precursor, because the reaction can be carried out under mild condition which is suitable for our module. In addition, **1** is commercially available. The process of [^{18}F]FES synthesis is outlined in Figure 1. The step (a) and (b) were performed in TRACERlab MX_{FDG}. There were only three points different between the [^{18}F]FES synthesis and the FDG synthesis as follows; the synthesis program, the regents and the cassette layout. The advantage of this method was that the synthesis of [^{18}F]FES and FDG can be performed using the same machine without a change to the hardware.

The fluorination of **1** was performed by the nucleophilic substitution reaction by $K^{18}F$ and accomplished with high yield (89.5 ± 0.8 %, n=5). The next hydrolysis step consists of two consecutive reactions. Römer reported two methods of the hydrolysis for their automated module: the pressure hydrolysis method using HCl and the multiple azeotropic evaporation method using hydrochloric acetonitrile [12]. They recommended the latter because it had an advantage of removing the acid and unreacted [^{18}F]fluoride at the same time. We tried this method, but the radioactivity in the reaction vessel varied between the different runs (40.0 ± 27.3 %, n=5). We suspected that the main reason for the inconsistency was decomposition of [^{18}F]FES during the azeotropic evaporation, though it might have depended on the module we used. As it seemed that the decomposition occurred under water rich condition, we speculated that maintaining acetonitrile/water ratio during the reaction would lead to high and reproducible

yield of [^{18}F]FES. In order to keep the acetonitrile component, we carried out the reaction using 90% hydrochloric acetonitrile in closed valve condition. In this condition, [^{18}F]FES was formed within 6 min after the start of hydrolysis at 95°C, and the yield reached the plateau level within another 4 min. Therefore, we decided the reaction time to be 10 min. In this way, most of the radioactivity in the reaction vessel was maintained during the hydrolysis. Figure 3 shows the chromatogram of the HPLC purification. The retention time of [^{18}F]FES was 16.2 min, which corresponded to that of unlabeled authentic FES. The chromatogram demonstrated that the [^{18}F]FES synthesis was accomplished with a yield of 76.4 ± 1.9 % based on the crude [^{18}F]FES solution (n=5). It indicated that the fluorination and the hydrolysis were achieved properly. This method realized sufficiently high and reproducible yields for clinical use as we had intended. The volume of the crude [^{18}F]FES solution was about 3 ml without the reagent F and the pH of this solution was 8. The purified [^{18}F]FES was stable when the mobile phase of HPLC contained 30% ethanol.

The formula of the final product was also examined. Several researchers provided the final product of [^{18}F]FES as a ethanol/saline solution, probably because of easy preparation from HPLC eluate and high lipophilicity of [^{18}F]FES. However, ethanol causes unacceptable side effects in some patients. Thus, we attempted to prepare the final product as saline solution. However, the radiochemical purity of the product declined rapidly. The percentage of intact [^{18}F]FES was 87% at 2 h (Figure 4). It indicated that ethanol might be working not only as solvent but also as a radical scavenger. When sodium ascorbate was added as a radical scavenger instead of ethanol to prevent the decomposition, the radiochemical purity of [^{18}F]FES in saline was over 98% after 6 h. In the filtering process, about 10 % of the radioactivity based on input [^{18}F]fluoride was trapped on the membrane filter. Fortunately, the final product through the filter was not trapped much in the final glass vial and the polyethylene syringe used for injection. The radioactivity trapped on the filter could be eluted by 70% ethanol and was

confirmed as intact [^{18}F]FES. Even though our formula causes the decrease of the final product in the filtering process, however it has the advantage of avoiding the side effects in clinical use. The final product was obtained with a yield of 42.4 ± 3.2 % based on input [^{18}F]fluoride (decay-corrected, n=5). The specific activity was calculated by the analytical HPLC system (the detection limit was 0.1 μg/ml) and the value was more than 111 GBq/μmol.

The total preparation time was 88.2 ± 6.4 min. The automated synthesis on TRACERlab MX_{FDG} was completed in 50 min. The HPLC purification took 20 min and another 20 min was required for the formulation process. If the final product was prepared in ethanol/saline solution like in other reports, the ethanol/water solution can be used as the mobile phase in purification and the following formulation process can be omitted.

CONCLUSION

In this study, we demonstrated that [^{18}F]FES for clinical use can be prepared with high and reproducible yield using a commercial FDG synthesizer. The merit of our method is that [^{18}F]FES can be easily prepared in a PET institution without a chemist because it does not require expertise in chemistry. Our preparation system enables routine use of [^{18}F]FES in clinical PET centers.

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FIGURE LEGENDS

Figure 1. Scheme of 16α-[^{18}F]fluoro-17β-estradiol synthesis for clinical use starting from 3-*O*-methoxymethyl-16,17-*O*-sulfuryl-16-epiestriol (**1**).

Figure 2. Schematic diagram of the [^{18}F]FES preparation system for clinical use.

Figure 3. The chromatograms of the crude [^{18}F]FES at the HPLC purification. Top: radioactivity, Bottom: UV absorbance at 280 nm. The retention time of [^{18}F]FES was 16.2 min, which corresponded to the authentic unlabeled 16α-fluoro-17β-estradiol.

Figure 4. The stability of [^{18}F]FES in the final product for clinical use. [^{18}F]FES was rapidly degraded in saline without sodium ascorbate.

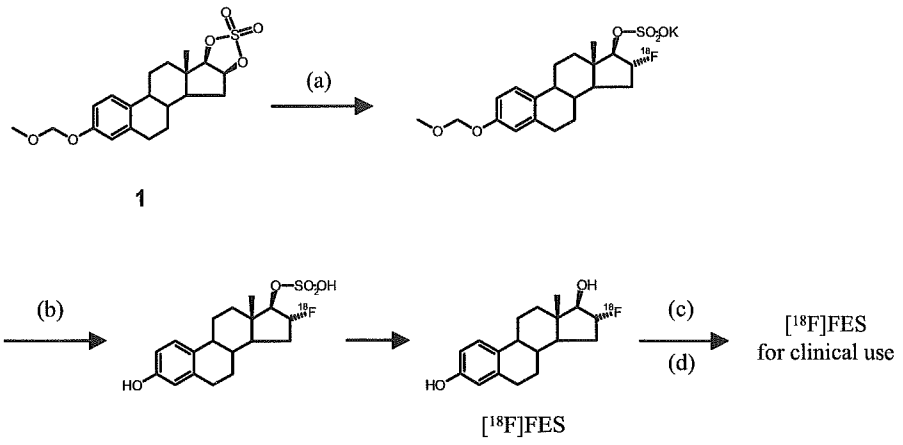
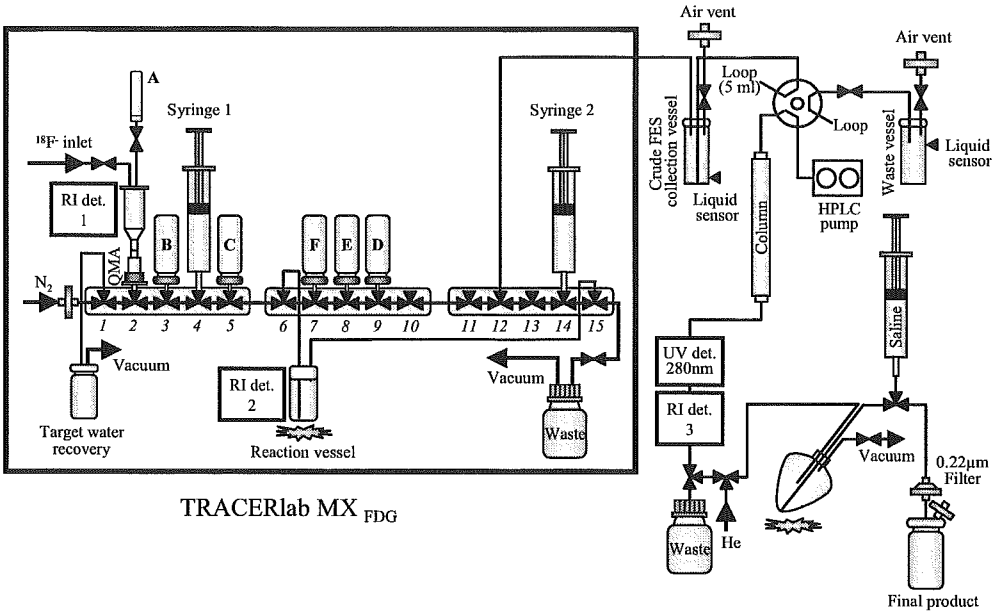

(a)
1
(b)
(c)
(d)
[18F]FES
for clinical use
[18F]FES
(a) Fluorination
(b) Hydrolysis
(c) HPLC purification
(d) Formulation

Figure 1



TRACERlab MX $_{FDG}$

Figure 2

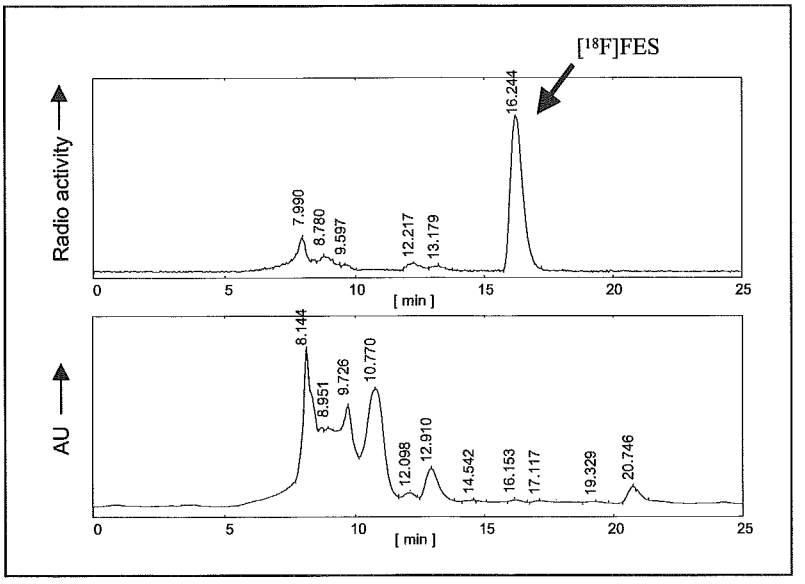

[18F]FES
Radio activity
7.990
8.780
9.597
12.217
13.179
16.244
0
5
10
15
20
25
[min]
AU
8.144
8.951
9.726
10.770
12.098
12.910
14.542
16.153
17.117
19.329
20.746
0
5
10
15
20
25
[min]

Figure 3

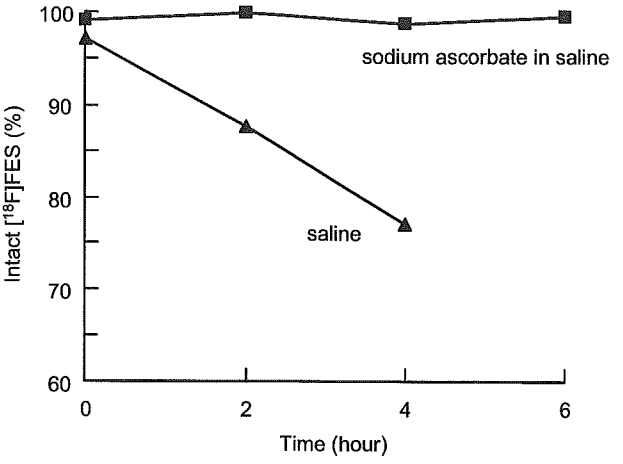

100
90
80
70
60
Intact [18F]FES (%)
sodium ascorbate in saline
saline
0
2
4
6
Time (hour)

Figure 4

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