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SYNTHESIS OF 16α-[^{18}F]FLUOROESTRADIOL-3,17β-DISULPHAMATE

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SUMMARY

The synthesis of 16α-[^{18}F]fluoroestradiol-3,17β-disulphamate ([^{18}F]FESDS) is described. 16α-[^{18}F]Fluoroestradiol ([^{18}F]FES) is converted using excess sulphamoyl chloride in absolute acetonitrile in the presence of Kryptofix 2.2.2 and potassium carbonate using an automatically operating module. The required time for the synthesis related to end of bombardment is 3h, the maximum yield is 6%, and the maximum decay-corrected yield is 20%. The radiochemical purity of [^{18}F]FESDS is > 99%. The specific radioactivity of [^{18}F]FESDS is found to be between 150 and 200 GBq/μmol.

Key words: 16α-[^{18}F]Fluoroestradiol-3,17β-disulphamate, ^{18}F-labelled tracer, positron emission tomography, sulphamoylation, HPLC purification

INTRODUCTION

The development of new specific oncological ^{18}F-labelled tracers for the positron emission tomography (PET) is an emerging area [1], especially, for diagnosing endocrine-dependent breast cancer [2] and breast cancer metastases [3], 16α-[^{18}F]fluoroestradiol ([^{18}F]FES) has proved to be the PET tracer of choice [4]. In order to make the new PET tracer accessible to the nuclear physicians at our location, a synthesis , elaborated in the USA [5], was modified at our PET

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centre [6] and integrated into an automatic module [7]. Thus, [^{18}F]FES can be reliably produced in GBq amounts of high specific radioactivity.

Recently, we have found that estradiol-3,17β-disulphamate and 16α-halogen-estradiol-3,17β-disulphamates are highly active steroid sulphatase inhibitors. Estrogen sulphatases *in vivo* are responsible for the deconjugation of estrogen sulphate. This deconjugation is necessary because only free estrogen can exercise its biological function. Free estrogens are mitogens and promote the growth of endocrine-dependent tumours. As steroid sulphatase is particularly prevalent in breast cancer tissue [8], sulphatase inhibitors that prevent the formation of free estrogen should be suitable for chemotherapeutic intervention in breast cancer [9]. When using 16α-[^{18}F]fluoroestradiol-3,17β-disulphamate ([^{18}F]FESDS) on the other hand, there should be the chance to image sites of high sulphatase activity. For such examinations [^{18}F]FESDS had to be made available. The access to [^{18}F]FES by a rapid and reliable production method [7] was a good starting point to accomplish such a project.

In this paper we describe the conditions which are suited to successfully convert [^{18}F]FES into chemically and radiochemically pure [^{18}F]FESDS.

RESULTS and DISCUSSION

A simple phase-transfer method for the preparation of unsubstituted sulphamate esters , H_2N-SO_2-OR , where R can be aliphatic or aromatic, was described by Spillane et al. [10]. These authors were the first to use solid sodium carbonate as a deprotonating medium. We transferred this successful method for the first time to steroid alcohols [11].

Sulphamoyl chloride (H_2N-SO_2-Cl, SCl) [12], a solid moisture-sensitive compound, was used as reagent. It formed three sulphamates with 16α-fluoro-estradiol (FES). These new compounds - 16α-fluoro-estradiol-3-sulphamate (FES3S), 16α-fluoro-estradiol-17β-sulphamate (FES17S), and 16α-fluoro-estradiol-3,17β-disulphamate (FESDS) - were first synthesized and characterized in our laboratory [11].

The sulphamoylation reaction of FES or other 3,17ξ-diols yielded mixtures of sulphamates but in all the cases investigated the formation of the 17ξ-sulphamate was favoured. When using excess SCl, almost all the diol could be converted into diol-3,17ξ-disulphamate.

To separate a sulphamoylation mixture, preparative reversed-phase (RP) HPLC was used. Usually, pure acetonitrile was the eluent [11] but, 55% aqueous ethanol proved to be a better eluent capable of separating FES and all the sulphamates of FES in an excellent manner. Because the diol-monosulphamate is more polar than the diol, and the dioldisulphamate is more polar than a monosulphamate, FESDS was eluted from an RP column before the monosulphamates. The retention times of FES, FES3S, FES17S, and FESDS are listed in Table 1.

The new sulphamates were characterized by melting point, mass, UV, and ^{13}C-NMR spectroscopy, and by thin-layer chromatography (TLC) [11]. TLC was also used in the radioactive experiments described here. Therefore, R_f values were also noted in Table 1.

Table 1 : Retention times and R_f values of FES, FES-monosulphamates and FES-disulphamate

Compound [a]	Retention Time [min] [b]	R_f Value [c]
FES	30.5	0.32
FES3S	22.5	0.17
FES17S	21.0	0.27
FESDS	17.0	0.21

a) Abbreviations see text
b) SP 250/10 NUCLEOSIL 100-7C_{18} from Macherey & Nagel; 55% ethanol as eluent at a flow of 1.5 mL/min
c) Silica gel; toluene/ethyl acetate (3:1) as solvent

An automated synthesis should be straightforward. Liquid-liquid extractions and adding solids are difficult in a module-assisted procedure. Therefore, we tested the usefulness of the soluble 2,6-di-tert.butyl-4-methyl pyridine (DBMP) instead of sodium carbonate as a base.

The usefulness of DBMP had already been established in non-radioactive experiments [11]. DBMP worked even if used in sub-stoichiometric amounts in relation to FES. Milligram amounts of FES in absolute acetonitrile were converted into FESDS at room temperature by using a twentyfold excess of SCl

in the presence of sub-stoichiometric amounts of DBMP, the reaction being complete in a few minutes.

Conversion of [^{18}F]FES in the presence of DBMP

In the radioactive experiments, solutions of DBMP (1 mg) and SCl (10 mg) in acetonitrile were added to column-purified dry [^{18}F]FES. The solution was stirred at room temperature. Samples were taken from time to time and analysed, using analytical radio-HPLC. But contrary to the optimum non-radioactive conditions mentioned above, no conversion of [^{18}F]FES was detected. Only some negligible conversion was observed after the temperature had been increased. However, complete conversion of the [^{18}F]FES was shown by analytical radio-HPLC when the reaction batch was evaporated to dryness at 70° C and ethanol was added to the residue. Mainly polar products (about 75%), but only about 25% of the desired [^{18}F]FESDS, were found in the analytical radio-chromatogram. Further experiments confirmed these results and proved that complete conversion of [^{18}F]FES had taken place in the process of evaporation. If a reaction batch treated in this way was injected into a semi-preparative RP column and then eluted with 55% aqueous ethanol, a typical radiochromatogram was obtained as shown in Figure 1 . Peak collection, radioactivity measurement and decay correction provided precise data on the radioactivity distribution:

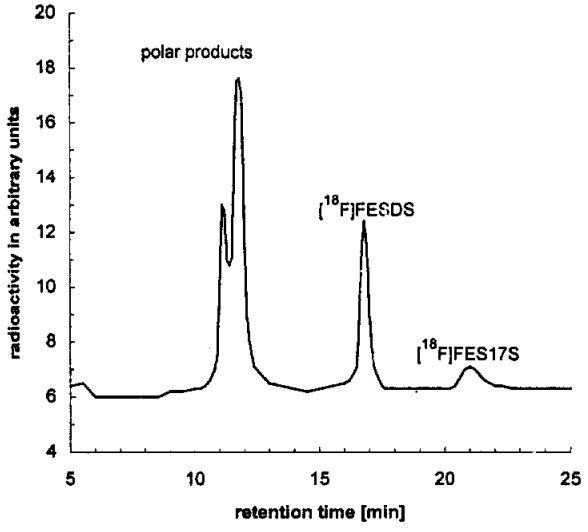

Figure 1 : The radiochromatogram of a reaction mixture prepared by sulphamoylation of [^{18}F]FES in the presence of DBMP

polar products (69.6%), [^{18}F]FESDS (25.9%), and another product with a retention time of 21 min, probably [^{18}F]FES17S (4.5%). A check of the collected eluate of the [^{18}F]FESDS peak showed a radiochemical purity of > 99%.

Contrary to non-radioactive experiments, in radioactive experiments DBMP cannot be used in sub-stoichiometric amounts. We tried to reduce the formation of polar by-products by lowering the amount of DBMP to 0.1 mg. Even with this small amount, [^{18}F]FES was completely converted without increasing the yield of [^{18}F]FESDS. Further reduction of DBMP finally led to [^{18}F]FES not being converted at all.

Conversion of [^{18}F]FES in the presence of other base

Dimethyl formamide was recommended in the literature [13] as a good solvent for sulphamoylation because it also acts as a base. We investigated the sulphamoylation reaction of [^{18}F]FES in a solvent mixture of acetonitrile and dimethyl formamide, first at room temperature, then at 60°C. No conversion of [^{18}F]FES occurred. Removal of the solvent at 100°C and adding ethanol to the residue resulted in the quantitative conversion of [^{18}F]FES into polar products. No trace of [^{18}F]FESDS could however be detected.

Quantitative conversion of [^{18}F]FES into polar products also occurred when only small amounts of sodium carbonate or potassium carbonate were used. This result showed that the occurrence of undesirable by-products in the previously described experiments cannot be attributed to any special property of DBMP. It was surprising, on the other hand, that in the non-radioactive experiments for the preparation of FESDS [11] no polar product was formed in the presence of excess sodium carbonate.

Conversion of [^{18}F]FES in the presence of Kryptofix 2.2.2 / K_2CO_3

Mixtures of Kryptofix 2.2.2. (K222) and potassium carbonate in absolute acetonitrile have proved to be useful in nucleophilic fluorination reactions [14-16]. For preparing [^{18}F]FDG [17] and [^{18}F]FES [7], we have made use of a solution containing 40 μmol K222 and 20 μmol K_2CO_3 per mL (K222/K_2CO_3). Now we examined whether this solution can be also successfully applied in the sulphamoylation reaction of [^{18}F]FES.

K222/K_2CO_3 solution (1.5 mL) was added to column-purified [^{18}F]FES. After careful evaporation of the solvent to dryness, a solution of SCl in acetonitrile

was added, and this reaction mixture was stirred at 70°C. Surprisingly, a sample taken after 5 min already showed a 75% conversion of [^{18}F]FES. After work-up, [^{18}F]FESDS and polar products were present in a ratio of about 1 : 1 as demonstrated by analytical HPLC. The same ratio was found after separation on a semi-preparative RP column. A radiochromatogram is shown in Figure 2. The reproducible preparation of [^{18}F]FESDS was confirmed by further experiments. The conversion level was between 50 and 60%.

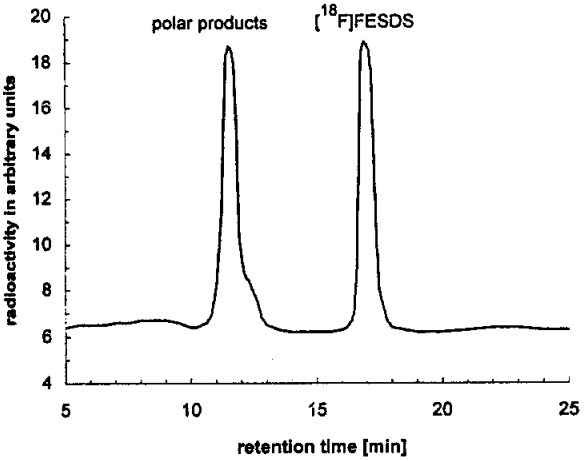

Figure 2: The radiochromatogram of a reaction mixture prepared by sulphamoylation of [^{18}F]FES in the presence of K222/K_2CO_3

Compared with the process using DBMP, sulphamoylation of [^{18}F]FES in the presence of K222/K_2CO_3 showed some advantages. (1) Sulphamoylation occurred when the solution was being heated. (2) The desired [^{18}F]FESDS was obtained in a significantly higher yield. (3) The process was reproducible.

These advantages were probably attributable to the special properties of the combination of K222 and K_2CO_3. Whereas heterogeneous K_2CO_3 alone was only effective in the evaporation process and yielded nothing but polar products, in combination with K222 it became a moderate homogeneous base. This moderation effect depended on K222 concentration, which was found to be optimum between 10 and 20 μmol/mL. But even under these conditions, the occurrence of polar products could not be ruled out.

The method with K222/K_2CO_3 was then transferred into an automatically operating module. The experiments were carried out at a higher radioactivity level. The same module was used as for preparing [^{18}F]FES. After synthesis of [^{18}F]FES according to [7], the module had to be cleaned and prepared for the sulphamoylation reaction which was then carried out as described above. Finally, the reaction mixture was separated on a semi-preparative RP-HPLC column.

The results of three typical module-assisted experiments are summarized in Table 2. The yield of [^{18}F]FESDS related to [^{18}F]FES was about 50%. The maximum yield of [^{18}F]FESDS related to the starting radioactivity at t = 0 was 6% (real yield) and 20% (decay-corrected), respectively. The total time for producing [^{18}F]FESDS was about 3h. At the end of synthesis [^{18}F]FESDS was available at up to 1000 MBq.

Table 2: Results of three module-assisted experiments

Expt.	Irradiation time [min]	Starting activity [MBq] a)	[^{18}F]FES		[^{18}F]FESDS	
			Time [min] b)	$Y_{corr.}$ [%] c)	Time [min] b)	$Y_{corr.}$ [%] c)
1	15	13150	66	39.8	187	19.7
2	30	29500	62	33.8	189	16.5
3	20	22150	62	37.0	190	19.2

a) Radioactivity at the start of the synthesis (t = 0)
b) Time between t = 0 and the moment of availability of the radiotracer
c) Yield of the radiotracer related to t = 0

Chemical and radiochemical purity, specific radioactivity

The high chemical and radiochemical purity of [^{18}F]FES and its high specific radioactivity were already shown by analytical HPLC [7]. Now, the purity of the [^{18}F]FESDS had to be determined.

Some results from Expt. 2 (Table 2) were represented in Figure 3. There are four chromatograms; a UV chromatogram of a reference solution containing FES and FESDS (Figure 3A), a radiochromatogram of the raw product before it was injected into the semi-preparative RP column to be separated (Figure 3B), a radiochromatogram of the collected eluate of the [^{18}F]FESDS peak (Figure 3C), and a UV chromatogram of the same solution (Figure 3D).

According to Figure 3A the retention times of FESDS and FES were 15.7 min and 19.0 min, respectively. When the raw product was analysed (Figure 3B), a peak representing polar products (30%) was found at 10.6 min, a large peak (58%) was eluted at 16.0 min, and a small peak (7%) at 19.2 min. In Figure 3C,

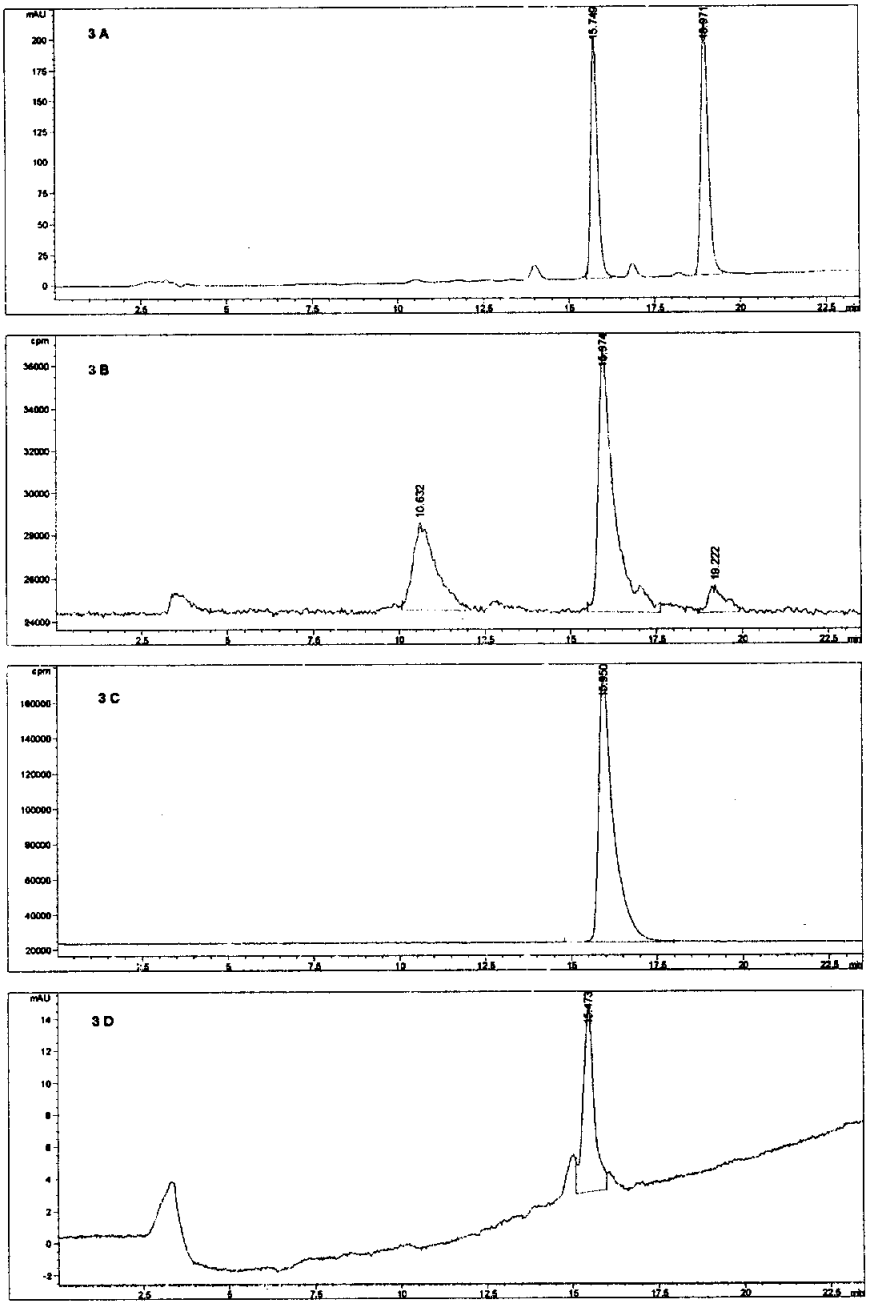

Figure 3 : Analytical HPLC Chromatograms
3A – UV chromatogram of a reference solution containing FES and FESDS
3B – Radiochromatogram of the impure product of Expt. 2 in Table 2
3C – Radiochromatogram of the column-purified [^{18}F]FESDS
3D – UV chromatogram of the column-purified [^{18}F]FESDS

the successful HPLC separation was demonstrated with the peak at RT = 16.0 min representing [^{18}F]FESDS of high chemical and radiochemical purity(> 99%). The named distribution and the radiochemical purity of [^{18}F]FESDS were confirmed by radio TLC. The impure product showed three spots and the following radioactivity distribution: polar products (29%), [^{18}F]FESDS (65%), [^{18}F]FES (6%). The R_f values found were 0.18 for [^{18}F]FESDS and 0.32 for [^{18}F]FES. The pure [^{18}F]FESDS solution showed one and only peak (R_f = 0.18) with a radiochemical purity of > 99%.

The specific radioactivity of the ^{18}F-labelled compound was highest the greater the starting radioactivity used [7]. Only solutions of pure [^{18}F]FESDS from the experiments in Table 2 were investigated. We injected 100 µl into the analytical HPLC system. A small peak due to FESDS was found at 216 nm (Figure 3D). The peak mass was determined and values for the specific radioactivity of between 150 and 200 GBq/µmol were obtained. The identity of reference FESDS and the product synthesized in the module was confirmed by mass spectroscopy (m/z 448).

EXPERIMENTAL

Chemicals: Sulphamoyl chloride was synthesized according to a modified procedure of Appel and Berger [12]. The synthesis of FESDS is described in [11]. Kryptofix 2.2.2 and DBMP were purchased from Merck.

Preparing the no-carrier-added (n.c.a.) [^{18}F]HF: The Rossendorf PET Centre is equipped with an IBA CYCLONE 18/9 cyclotron. [^{18}F]HF is produced by the ^{18}O(p,n)^{18}F reaction using enriched [^{18}O]H_2O (1.5 mL, 95%) as target. For the optimization experiments only limited amounts of [^{18}F]HF were necessary. Therefore, the following irradiation conditions were usually used:

- Ion beam current on the target: 30 µA (18 MeV protons)
- Irradiation time: 5 minutes

After end of bombardment, the irradiated water was transferred from the target to the module, and here the starting radioactivity at t = 0 was measured .

Preparing the n.c.a. [^{18}F]FES: The synthesis was carried out in a module (Nuclear Interface, Germany) which was modified in terms of program and hardware [7]. A semi-preparative RP column (SP 250/10 Nucleosil 120-7 C_{18},

Macherey & Nagel, Germany) served for separation of [^{18}F]FES. Aqueous ethanol (55%) was used in order to transfer the impure radioactive product from the reaction vessel into the column and then to elute [^{18}F]FES isocratically at a flow of 1.5 mL/min.

Optimization experiments to synthesize [^{18}F]FESDS : About 100 – 500 MBq of n.c.a.[^{18}F]FES dissolved in about 2 mL 55% aqueous EtOH was placed in a bulb. After adding a solution of a base (either DBMP: 0.1 mg in 0.5 mL MeCN or K222/K_2CO_3: 22.5 mg K222 and 4.2 mg K_2CO_3 in 1.5 mL MeCN), the solvent was removed on a rotary evaporator. A solution of 10 mg SCl in 2 mL MeCN was added to the absolutely dry reaction batch. The bulb was stirred at room temperature or at higher temperature (up to 70 °C). Samples of 10 µl each were investigated in an analytical HPLC system (Hewlett-Packard, series 1050, diode array detector 1040 M, radioactivity detector A 100 from Canberra Packard. Column: ET 125/8/4 NUCLEOSIL 120-5 C_{18} from Macherey & Nagel. EtOH-water mixtures were the mobile phase at a flow of 0.5 mL/min with a linear gradient: 0 min – 30% EtOH, 20 min – 60% EtOH, 30 min – 100% EtOH). To work up the reaction batch, the solvent was removed on a rotary evaporator and 55% aqueous EtOH (0.6 mL) was added. The clear solution (0.5 mL) was injected into a semi-preparative HPLC system (Merck-Hitachi, pump L-6200A, a Rheodyne injector with a 0.5 mL loop, an RP column SP 250/10 NUCLEOSIL 100-7C_{18} (Macherey & Nagel, Germany) and a diode array detector L-4500 DAD). The column was eluted with 55% aqueous EtOH at 1.5 mL/min. After UV measurement at λ = 275 nm , the eluted solution was measured in a radioactivity detector (Canberra Packard A 120). Each solvent peak was collected and the radioactivity measured in an ionisation chamber (Nuklear Medizintechnik Dresden, Germany).

Module-assisted experiments to synthesize n.c.a.[^{18}F]FESDS: [^{18}F]FES was synthesized as described above. After cleaning the module, the collected eluate of [^{18}F]FES (1 - 3 GBq in 3 - 5 mL of 55% aqueous EtOH) was transferred into the reaction vessel of the module in which the following four solutions were already stored in four vials:

Solution I K222 (22.5 mg) and K_2CO_3 (4.2 mg) in 1.5 mL 86% aqueous MeCN
Solution II SCl (15 – 20 mg) in 1.5 mL absolute MeCN
Solution III 22% aqueous EtCH (4 mL)
Solution IV absolute MeCN (12 mL)

The following operating steps were carried out:

- Addition of solution I and 4 mL of solution IV and drying the reaction mixture at 100 °C for 4 min.
- Addition of only 4 mL of solution IV and drying the reaction mixture at 100 °C for 4 min.
- Repetition of the last drying process and temperature reduction to 70 °C.
- Addition of solution II. The reaction mixture is stirred for 5 min at this temperature.
- Evaporation of the solvent within 1 min. Heating is switched off.
- Addition of solution III and waiting for 1 min.
- Transferring the solution into the injection loop.
- Injection of the solution into the column. Elution using 45% aqueous EtOH.
- Collection of the eluate of the [^{18}F]FESDS peak (RT = 25 min).
- Measuring the radioactivity of [^{18}F]FESDS.

*Radio-TLC :*Samples of the impure product solution and the collected eluate of the [^{18}F]FESDS were developed on a silica-gel plate (MERCK) in toluene/ethyl acetate (3:1). For visualizing the chromatogram, the TLC plate was contacted with an imaging plate (IP-BAS-III, 20x25, Fuji) for 15 s . The distribution of radioactivity on the plate was recorded by an imaging analyzer (BAS 2000, Fuji).

CONCLUSIONS

Sulphamoylation of [^{18}F]FES in the presence of K222/K_2CO_3 is a rapid and reproducible procedure. It is also applicable as a module-assisted procedure.The whole synthesis of [^{18}F]FESDS in a module-assisted way is composed of five steps:

- Module-assisted synthesis of [^{18}F]FES according to [7].
- Purifying [^{18}F]FES by RP-HPLC (see also [7]).
- Purifying the module.
- Module-assisted synthesis of [^{18}F]FESDS.
- Purifying [^{18}F]FESDS by RP-HPLC.

Starting with high levels of radioactivity, [^{18}F]FESDS can be prepared in amounts up to 5 GBq using this way.

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