PART I

FLUORINE-18 LABELED RADIOPHARMACEUTICALS

CHAPTER 1

SYNTHESIS OF [^{18}F]-FLUORODEOXYGLUCOSE ([^{18}F]FDG)

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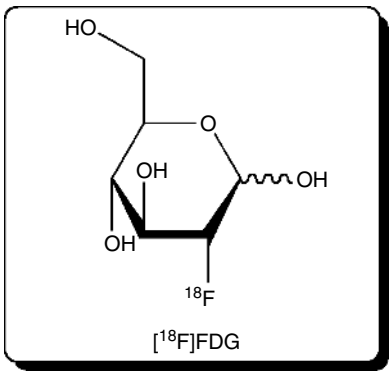

1 INTRODUCTION

Positron emission tomographic (PET) imaging provides a noninvasive, accurate diagnostic method of imaging and detecting possible diseases at a cellular, molecular, and tissue level. PET has a proportional relationship between the tissue intensity on the tomographic image and the actual radiopharmaceutical concentration in tissue [1]. [^{18}F]Fluorodeoxyglucose ([^{18}F]FDG) is a glucose analog and is known as the "work horse" of PET simply because of the multiple modalities for application. The implications of abnormal glucose metabolism are vital in evaluating a variety of diseases, and the applications of this positron emitting radiotracer are great. [^{18}F]FDG is preferentially taken up into cells with high metabolic activity by specific glucose transporters (GluT) and phosphorylated by hexokinase. It becomes metabolically trapped in the cell because [^{18}F]FDG-6-phosphate is not a substrate for glucose-6-phosphate isomerase, the enzyme that metabolizes glucose, and therefore cannot be broken down. During radioactive

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decay, ^{18}F decays to ^{18}O, allowing the decayed product, 2-^{18}O-deoxyglucose -6-phosphate, to enter the normal glucose metabolic pathway.

The first synthesis of [^{18}F]FDG was based on a direct electrophilic substitution reaction by Wolf et al. in 1976 [2,3]. This fluorination reaction by electrophilic substitution refers to the addition of fluorine atoms across a double bond, producing a difluoro derivative of the parent compound [4]. However, the synthesis of [^{18}F]FDG by nucleophilic substitution fluorination (Fig. 1) proved to be a more efficient method of producing [^{18}F]FDG in higher, more reproducible yields. In 1986, Hamacher et al. utilized mannose triflate, as the precursor, and Kryptofix-[2.2.2] dissolved in acetonitrile, as a catalyst, substituting the trifluoromethane sulfonate leaving group with [^{18}F]fluoride to form [^{18}F]FDG [5]. This method of generating [^{18}F]FDG has allowed for widespread use in clinical imaging [6] in neurology [7–9], oncology [10–15], and cardiology [16]. It has been significantly studied in numerous oncology studies, including lung cancer, lymphoma, and colorectal cancer [1]. Because [^{18}F]FDG is a marker for hexokinase activity and glucose metabolism, this PET radiotracer has become a valuable tool in tumor detection, staging, and treatment evaluation because most tumors are marked by increased glucose uptake [17]. New innovative applications of [^{18}F]FDG have been applied recently as well. In 2002, Chacko et al. investigated the possible application of [^{18}F]FDG in evaluating infections, such as osteomyelitis, infected orthopedic hardware, and deep-seated soft tissue infections, and concluded that [^{18}F]FDG was an effective imaging modality in the assessment of patients with suspected infections. However numerous the possible uses for [^{18}F]FDG are, it is important to note that [^{18}F]FDG is a nonspecific radiotracer for imaging disease. This chapter provides a brief overview of the radiochemical synthesis and clinical use of [^{18}F]FDG, but because of the enormous body of literature available on [^{18}F]FDG, a detailed discussion is beyond the scope of this chapter.

2 SYNTHESIS PROCEDURES

CAUTION: All radiochemical syntheses must be carried out using the appropriate equipment in a facility authorized for the use of radioactive materials. Personal protective equipment must be worn, and all local radiation safety laws followed.

2.1 Preliminary Steps

A General Electric Medical Systems (GEMS) TRACERlab MX_{FDG} module [19] was used for the synthesis of [^{18}F]FDG at the University of Michigan Cyclotron and Radiochemistry Facility. The preliminary steps for the [^{18}F]FDG synthesis were performed before the transfer of [^{18}O]H_2O into the synthesizer. Initial tests run by the MX_{FDG} module ensured that the heater, syringe actuators, compressed air, vacuum pump, and nitrogen flow were all properly working.

The single-use TRACERlab MX_{FDG} cassette (Fig. 2) [20], manufactured by GE Healthcare, was assembled in a Horizontal LAF Hood. The cassette came prepackaged with two tC18 cartridges and one alumina-N cartridge. The cassette

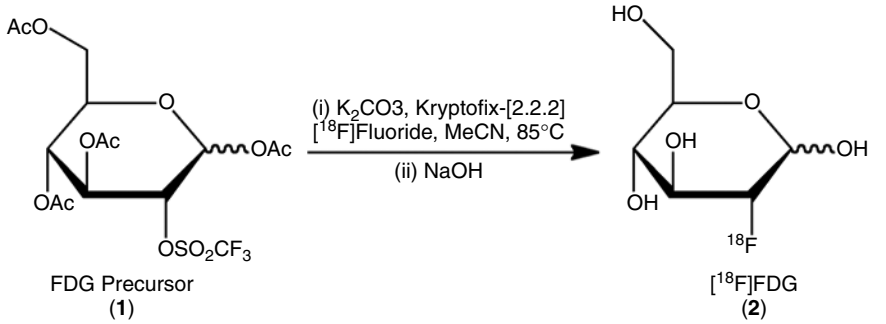

FIGURE 1 Synthesis of $[^{18}F]$FDG by nucleophilic aliphatic substitution on tetraacetyl mannosyl-2-triflate.

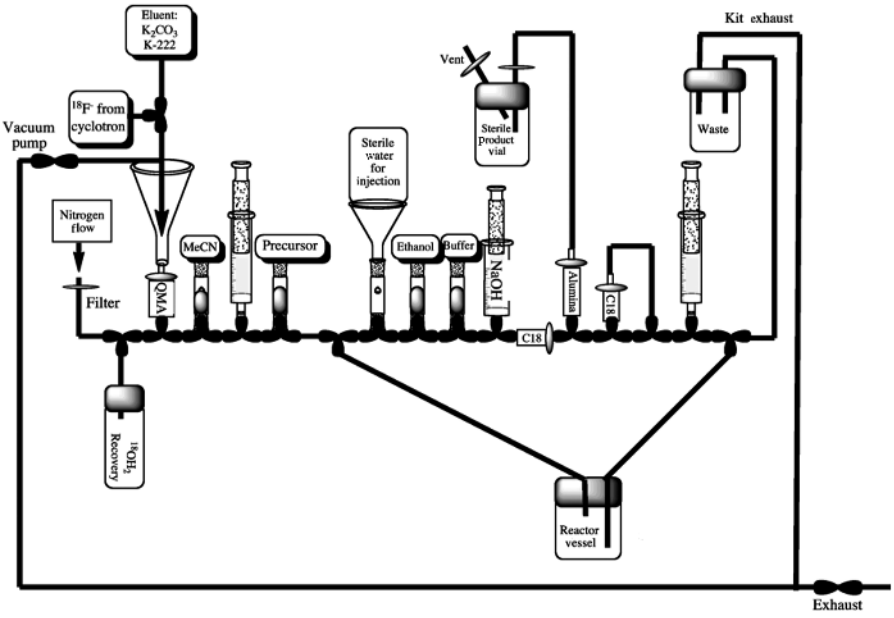

FIGURE 2 Single-use cassette for manufacture of $[^{18}F]$FDG.

was assembled per manufacturer recommendations, which included attaching one Millipore Millex-FG sterilizing filter [21] to the nitrogen inlet connector tubing, a conical column reservoir [22] to collect the $[^{18}O]H_2O$ transferred from the cyclotron to the cassette, a QMA Sep-Pak Light Accell Plus cartridge [23], two single-use 30-mL syringes [24], ABX manufactured mannose triflate [25] precursor, and the ABX synthesizer reagent kit [26] containing predispensed volumes of acetonitrile, ethanol, neutralization solution, sodium hydroxide, Kryptofix-[2.2.2]/potassium carbonate solution, and sterile water for injection. The neutralization solution [26], with an approximate pH of 6.4, was a mixture of citrate

buffer with hydrochloric acid, resulting in a composition of 23.5 mg of disodium hydrogen citrate-0,5-hydrate (extra pure), 144.4 mg of trisodium citrate-2-hydrate (extra pure), 5 mL of pure water, and 1 mL of HCl 2 N for one synthesis, and was manufactured by ABX.

Once the cassette was mounted to the MX_{FDG} module, additional steps performed included hooking the syringes onto the syringe actuators, dissolving the precursor in 3.5 mL of acetonitrile, pressurizing the reagent vials used during synthesis, and rinsing the single-use cassette with acetonitrile.

2.2 Production of [^{18}F]Flouride

A GEMS PETtrace cyclotron [19] was used to generate [^{18}F]fluoride by the $^{18}O(p,n)^{18}F$ nuclear reaction using a [^{18}F]fluoride target [27]. Approximately 2 mL of [^{18}O]H_2O [28] was loaded into the [^{18}F]fluoride target and bombarded at 40 μA for 25 min to generate approximately 1 Ci of activity (37 GBq).

2.3 Azeotropic Drying of [^{18}F]Fluoride

After bombardment, the [^{18}F]fluoride was transferred to a GEMS TRACERlab MX_{FDG} synthesizer [19] in approximately 2 mL of [^{18}O]H_2O [28], where it was passed through an anion exchange cartridge [23]. The fluoride ions were trapped on the cartridge and the [^{18}O]H_2O was recovered.

Once the [^{18}O]H_2O was recovered, the [^{18}F]fluoride ions were eluted from the anion exchange cartridge into the reaction vessel using a 0.6 mL solution [26] containing 7 mg of potassium carbonate, 22 mg of Kryptofix-[2.2.2], 300 μL of water, and 300 μL of acetonitrile. In the reaction vessel, the solvents were evaporated at 95°C for 8.5 min under negative pressure and nitrogen flow. Throughout the evaporation process, 80 μL of acetonitrile was added three times to the reaction vessel during the evaporation stage to maintain the H_2O/MeCN azeotrope, totaling 240 μL of acetonitrile.

2.4 Synthesis of [^{18}F]FDG

During the preliminary steps of preparing the GEMS TRACERlab MX_{FDG} module for synthesis, 40.0 mg of mannose triflate precursor [25] was dissolved in 3.5 mL of acetonitrile [26]. Following drying of the fluoride, the precursor was added to the reactor vessel. A nucleophilic aliphatic substitution reaction occurred, in which the [^{18}F]fluoride ions replaced the trifluoromethane sulfonate leaving group, creating the 2-[^{18}F]fluoro-1,3,4,6-tetra-*O*-acetyl-D-glucose molecule. This reaction occurred at 85°C for 4 min in a closed reactor vessel.

2.5 Purification of [^{18}F]FDG

The tC18 cartridges used for hydrolysis and purification were rinsed with 10 mL of sterile water for injection [26]. Afterwards, both tC18 cartridges [20] were conditioned with 3 mL of ethanol and 22 mL of water during synthesis. The labeled solution was then diluted with 26 mL of water and passed through the first tC18 cartridge, which was used for hydrolysis. The 2-[^{18}F]fluoro-1,3,4,6-tetra-*O*-acetyl-D-glucose molecule was trapped on the cartridge, while all other solvents,

unreacted [^{18}F]fluoride ions, and polar byproducts were washed away into the external waste bottle. Kryptofix was trapped on this cartridge as well. The hydrolysis cartridge was washed three times with water; the first rinse was with 10 mL of water, the second with 10 mL of water, and the final with 23 mL of water.

Alkaline hydrolysis of the labeled compound occurred at room temperature, using 800 μL of 2 N sodium hydroxide. The sodium hydroxide passed through the tC18 cartridge, removing the four acetyl-protecting groups. [^{18}F]FDG was collected from the tC18 cartridge in water and pulled into a 30-mL syringe [24]. The neutralization solution [26] was added to the alkaline [^{18}F]FDG solution (10.8 mL) in order to provide a solution of physiological pH suitable for injection. The final formulation (16.8 mL) was further purified using a second tC18 cartridge [20] and an alumina-N cartridge [20], and passed through a sterile Millex-GS 0.22-μm filter [29] into a sterile dose vial [30], typically generating 500–600 mCi (18.5–22.2 GBq) of [^{18}F]FDG. The solution was then released for quality control (QC) testing. After the synthesis was complete, the cassette was rinsed with the remaining sterile water to reduce residual radioactivity in the single-use cassette before removal and disposal.

3 QUALITY CONTROL PROCEDURES

CAUTION: All radiopharmaceuticals produced for clinical use must have local regulatory approval before use in humans. Trained personnel must carry out quality control procedures, and each dose must meet all established quality control criteria before release to the clinic.

QC tests at the University of Michigan Cyclotron and Radiochemistry Facility for [^{18}F]FDG were carried out in accordance with the current US Pharmacopeia [31,32] and are summarized below. Data from QC testing for three repeat batches of [^{18}F]FDG are summarized in Table 1. Each of the three doses of [^{18}F]FDG met all established QC criteria.

3.1 Visual Inspection

The [^{18}F]FDG dose must be clear, colorless, and free of particulate matter. This must be inspected behind proper shielding. A PET L-block barrier system was used.

3.2 Radiochemical Identity and Purity

Radiochemical purity was determined by thin layer chromatography (TLC) using silica gel chromatography plates [33] and a Bioscan AR-2000 TLC scanner [34]. The plate was spotted with approximately 10 μg of FDG reference standard solution [35] and then spotted with a sample of [^{18}F]FDG dose. The FDG reference standard solution was prepared by dissolving 10 mg of 2-fluoro-2-deoxy-D-glucose [35] in 50 μL of acetonitrile and 50 μL of water. The developing solution [36], or mobile phase, contained 95% acetonitrile and 5% water. The plate was developed in this solvent system, dried using a warm laboratory hot

TABLE 1 QC Data for Three Repeat Runs of [^{18}F]FDG

QC Test	Release Criteria	Run 1	Run 2	Run 3
Yield mCi (GBq)	N/A*	528 (19.5)	530 (19.6)	507 (18.8)
Percentage yield (corrected for decay)	N/A*	68.3	66.8	69.3
Visual inspection	Clear, colorless	Clear, colorless	Clear, colorless	Clear, colorless
Radiochemical identity	Compare to standard	$rf_{stnd} = 0.40$ $rf_{FDG} = 0.40$	$rf_{stnd} = 0.384$ $rf_{FDG} = 0.390$	$rf_{stnd} = 0.32$ $rf_{FDG} = 0.32$
Radiochemical purity (%)	≥90	96.69	96.19	97.02
Residual solvent analysis	<0.5% Ethanol <0.04% Acetonitrile	0.004% Ethanol, 0.005% Acetonitrile	0.014% Ethanol, 0.008% Acetonitrile	0.023% Ethanol, 0.008% Acetonitrile
Dose pH	4.5–7.5	6.0	5.5	6.0
Residual Kryptofix-[2.2.2] (μg/mL)	≤50	<10	<10	<10
Sterile filter integrity test (psi)	>50	54	56	54
Radionuclidic identity ($t_{1/2}$) (min)	105–115	109	111	109
Endotoxin analysis (EU/mL)	≤10.9	<2.00	<2.00	<2.00
Sterility testing	No microbial growth in the product culture tube up to 14 d	Pass	Pass	Pass

*Release criteria not available.

plate [37] (temperature setting 5), and placed on the TLC scanner for analysis. The Bioscan TLC Scanner measured the radiochemical purity, which must be greater than or equal to 90%.

Radiochemical identity was determined by TLC and analysis of the retention factor (Rf). The retention factor of the standard was compared to the [^{18}F]FDG dose sample placed on the TLC plate. One plate was developed and dried, as described above, for both radiochemical purity and identity analysis. Once the standard became visibly brown after heating, the plate was removed and placed on the TLC scanner for analysis. The Rf was calculated for the hot [^{18}F]FDG sample. The "cold" FDG standard retention factor was calculated manually and compared with the [^{18}F]FDG sample, the radioactivity distribution of which is determined by use of the Bioscan AR-2000 TLC scanner [34]. The Rf value of

the [^{18}F]FDG, free [^{18}F]fluoride, and acetylated [^{18}F]FDG are about 0.45, 0.0, and 0.85, respectively [4].

3.3 Radionuclidic Identity

A Capintec CRC®-15R Radioisotope Dose Calibrator [38] was used to measure activities. A sample of the [^{18}F]FDG product was placed in the Capintec Dose Calibrator located behind an L-block lead shield and measured, noting both the time and activity. After a period of at least 15 min, time and activity was noted again. The half-life was calculated using Equation 1, which must be within the range of 105–115 min for the dose to be released to the clinic.

$$t_{1/2} = -\ln 2(\text{Time difference}/[\ln(\text{ending activity}/\text{starting activity})]) \quad (1)$$

3.4 Residual Solvent Analysis

Residual solvent analysis of [^{18}F]FDG was performed on a Shimadzu GC-2010 Gas Chromatograph [39]. Acetonitrile and ethanol were tested for concentration levels and compared with a standard solution in accordance with the US Pharmacopeia [31]. The standard solution contained known concentrations of acetonitrile and ethanol, 0.01% and 0.1%, respectively. A Restek Stabliwax with Integra-Guard 30 m × 0.25 mm GC column [40] was used with helium as the carrier gas. Concentration percentages were calculated, and no more than 0.5% ethanol and 0.04% acetonitrile can be found in the dose, according to the US Pharmacopeia [31]. Acetonitrile, a class 2 solvent, has a limit on concentrations of <410 ppm per patient per day, imposed by the ICH Harmonised Tripartite Guidelines [41]. Ethanol, a class 3 solvent, has a limit of <5000 ppm/day.

3.5 Dose pH

ColorpHast® nonbleeding pH strips [42] were used to analyze a small amount of [^{18}F]FDG dose. The specification range of the colorpHast nonbleeding pH strips used were 2.0–9.0, and the determination of pH was done by visual comparison of the pH strip with the reference card supplied with the strips. The pH must be within the range of 4.5–7.5 to meet the release criteria.

3.6 Residual Kryptofix-[2.2.2] Analysis

Kryptofix-[2.2.2] is a phase transfer agent used to facilitate the nucleophilic fluorination reaction for [^{18}F]FDG, and because of its toxicity, verification of the absence of Kryptofix-[2.2.2] in the dose must take place before its release [43]. Residual Kryptofix-[2.2.2] levels in [^{18}F]FDG were analyzed using the established spot test. Kryptofix standards [44] were prepared by serial dilution of a 1.0 mg/mL stock solution using deionized water. Both water (negative control) and a 50-μg/mL standard (positive control) were used in this test. Stock iodoplatinate reagent was prepared to create iodoplatinate indicator strips used in spot testing. This reagent was generated using 5 mL of water mixed with 0.26 g of chloroplatinic acid [45]. This solution was then mixed with 45 mL of

water and 5 g of potassium iodide [46] and diluted with an additional 100 mL of water. Precut strips of thin-layer silica gel [47] were immersed in the iodoplatinate reagent for 5–10 s. Once the strips had been completely saturated by the reagent, they were dried overnight and then stored at room temperature in a vented glass jar.

A sample of the [^{18}F]FDG dose was applied to the indicator strip using a single droplet. On the same strip, both the negative water control and the Kryptofix standard [44] (50 μg/mL) were applied and allowed to dry for 5–10 min. The three spots were analyzed, and a visual determination of the relative concentration of Kryptofix-[2.2.2] was made for the [^{18}F]FDG dose.

The presence of Kryptofix-[2.2.2] in the dose is confirmed by the appearance of a blue-black ring around a white circle of silica surrounded by a pink ring of unreacted reagent. At very low or no concentrations of Kryptofix, a faint pink core remains in the center of the white circle, with no visible blue-black ring. The dose must contain $<$50 μg/mL for the dose to be acceptable for release.

3.7 Sterile Filter Integrity Test

Validation of the integrity of the sterile filter going into the dose vial must also be performed before the release of the dose to the clinic. The filter integrity test is essential in testing [^{18}F]FDG product sterility, as the actual sterility testing results will not be available until 14 days later [4]. The Millex-GS sterile filter [29] from the [^{18}F]FDG synthesis was attached to the nitrogen supply through a calibrated regulator. The tip of the filter was submerged in water. The nitrogen flow was gradually turned on to increase the nitrogen pressure on the filter. The pressure was increased to greater than 50 psi, which is the acceptance criterion for the Millipore-GS filter. If the pressure reaches 50 psi or more without resulting in a stream of bubbles in the water, the filter is considered intact. If a steady stream of bubbles is seen before the minimum bubble point pressure is reached, the test fails and the dose is rejected.

3.8 Endotoxin Analysis

The Charles River Portable Testing System [48,49] was used to determine the endotoxin content in the [^{18}F]FDG doses. Limulus Amebocyte Lysate (LAL) test cartridges [50], purchased from Charles River, were used in this test. A volume of 3.9 mL of Endosafe LAL Reagent water [51] was pipetted into a test tube. A volume of 0.1 mL of the [^{18}F]FDG dose was then added to this tube, mixed for 10 s using a vortex [52], and 25 μL of the diluted [^{18}F]FDG sample was added to each well on the Charles River test cartridge. This was performed in accordance with the US Pharmacopeia [53]. The dose must contain $\leq$175 Endotoxin Units (EU) per dose.

3.9 Sterility Testing

Sterility testing must also be performed on all doses of [^{18}F]FDG produced to verify that the process by which each PET radiopharmaceutical is produced is consistently sterile and suitable for human use. Sterility testing was performed by

inoculating samples of [^{18}F]FDG into a fluid thioglycolate media (FTM) [54] and a soybean casein digest media (SCDM) [55]. SCDM is used for the detection of aerobic bacteria and fungi, while the general purpose of FTM is to detect common aerobic, facultative, and anaerobic microorganisms.

The [^{18}F]FDG medium was incubated along with positive and negative controls for 14 days. Incubation temperatures were 32°C and 22°C for FTM and SCDM, respectively, according to current USP guidelines [56] for sterility testing. The media was visually inspected on the third, eigth, and fourteenth days of the incubation test period and compared to the positive and negative controls. The positive control must show growth (cloudiness or turbidity) in the medium, and the [^{18}F]FDG samples and negative control must show no growth after 14 days of incubation in order to be considered sterile.

CHEMICAL ABSTRACTS NOMENCLATURE (REGISTRY NUMBER)

Mannose triflate (92051-23-5)
[^{18}F]Fluorodeoxyglucose ([^{18}F]FDG) (128441-61-2)
Carabonic acid, potassium salt (1:2) (584-08-7)
Sodium hydroxide (1310-73-2)
Acetonitrile (75-05-8)
Ethanol (64-17-5)
2-Fluoro-2-deoxy-D-glucose (29702-43-0)
Kryptofix-[2.2.2] (23978-09-8)
Chloroplatinic acid (16941-12-1)
Potassium iodide (7681-11-0)

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14. Hutchings M, Eigtved AI, Specht L. Crit Rev Oncol/Hematol 2004;52:19–32.
15. Larson SM, Schwartz LH. J Nucl Med 2006;47:901–903.
16. Machac J. Semin Nucl Med 2005;35:17–36.

17. Black NF, McJames S, Kadrmas DJ. IEEE Trans Nucl Sci 2009;56:2750–2758.
18. Chacko TK, Zhuang H, Stevenson K, Moussavian B, Alavi A. Nucl Med Commun 2002;23: 851–855.
19. GE Healthcare, USA.
20. TRACERlab MX_{FDG} Cassettes were purchased from General Electric Healthcare (part no. P5150ME), and used as received. The cassettes came prepackaged and assembled with two tC18 cartridges and one alumina-N cartridge.
21. Millex-FG sterile 0.20 μm filters were purchased from Millipore (part no. SLFG025LS) and used as received.
22. Conical Column 10mL Reservoir was purchased from ABX (part no. 9180) and used as received.
23. QMA-Light Sep-Pak® preconditioned cartridges (part no. K-920) were purchased from Advanced Biochemical Compounds (ABX) and used as received.
24. 30mL syringes were purchased from Fischer Scientific (part no. 309650) and used as received.
25. Mannose triflate ultrapure precursor was purchased from ABX (part no. 101.0040) and used as received.
26. Coincidence FDG18 Synthesizer Reagent Kit was purchased from ABX (part no. K-153TM) and used as received.
27. GEMS silver high-yield [^{18}F]fluoride target.
28. Virgin [^{18}O]H_2O was purchased from ABX, Amic, Rotem, or Medical Isotopes, and used as received.
29. Millex-GS sterile 0.22-μm filters were purchased from Millipore (part no. SLGSV255F) and used as received.
30. Sterile 30-mL dose vial was purchased from Hospira (part no. 5829-30), and used as received.
31. U.S. Pharmacopeia <823> Radiopharmaceuticals for positron emission tomography compounding. USP 32-NF 27; 2009.
32. U.S. Pharmacopeia Official Monographs/Fludeoxyglucose. USP 29-NF 24; 2006.
33. Whatman Thin Layer Chromatography Plates (part no. 4861-110) MK6F Silica Gel 60 Å, Dimensions 2.5 × 7.5 cm, layer thickness 250 μm.
34. Bioscan, Inc., USA.
35. The FDG reference standard solution contained both 2-fluoro-2-deoxy-D-glucose reference standard, purchased from Sigma Aldrich (part no. F5006) and used as received, and anhydrous acetonitrile, purchased from Acros (part no. 61096-1000) and used as received.
36. The TLC developing solution contained anhydrous acetonitrile, purchased from Acros (part no. 61096-1000) and used as received and MilliQ water, in concentrations of 95% and 5%, respectively.
37. Laboratory Hot Plate, manufactured by Corning, Model no. PC-200, and used at temperature setting 5.
38. Capintec, Inc., USA.
39. Shimadzu Corporation, USA.
40. GC Column: Restek Stabliwax with Integra-Guard; 30 m × 0.25 mm, purchased from Restek (part no. 10623-124).
41. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH).
42. EMD Chemicals Inc., USA (part no. 9578-3).
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44. Kryptofix–[2.2.2] was purchased from Acros (part no. 29195-0010) and used as received for preparation of reference standard.
45. Chloroplatinic acid was purchased from Acros (part no. 363590010) and used as received.
46. Potassium iodide was purchased from Acros (part no. 19379-0500) and used as received.
47. Thin-layer silica gel sheets were purchased from EMD Chemicals (part no. 57487), 20 × 20 cm sheets, silica gel 60 Å, and cut into squares 4 × 5 cm.
48. Charles River Laboratories, USA.
49. Endosafe Portable Testing System (model no. PTS 100), purchased and calibrated according to manufacture guidelines by Charles River Laboratories, USA.

50. Limulus Amebocyte Lysate (LAL) Test Cartridges (part no. PTS20F) purchased from Charles River Laboratories and used as received.
51. Endosafe Limulus Amebocyte Lysate (LAL) Reagent water (part no. W110) purchased from Charles River Laboratories and used as received.
52. VWR Vortexer 2 (model no. G-560), vortex speed setting 8.
53. US Pharmacopeia <85>. Bacterial endotoxins test. USP 32-NF 27, 2009.
54. Fluid Thioglycolate Media (part no. 220889) purchased from Beckon, Dickson and Company, USA, and used as received.
55. Soybean Casein Digest Media (part no. 221823) purchased from Beckon, Dickson and Company, USA, and used as received.
56. US Pharmacopeia <71> Sterility tests. USP 32-NF 27, 2009.