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An improved preparation of [^{18}F]AV-45 by simplified solid-phase extraction purification

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Abstract:

Amyvid (florbetapir f18, [^{18}F]AV-45, [^{18}F]**5**) was the first FDA approved positron emission tomography (PET) imaging agent targeting β-amyloid (Aβ) plaques for assisting the diagnosis of Alzheimer's disease. This work aimed to improve the [^{18}F]AV-45 ([^{18}F]**5**) preparation by using solid-phase extraction (SPE) purification. [^{18}F]AV-45 ([^{18}F]**5**) was synthesized by direct nucleophilic radiofluorination of *O*-tosylated precursor (1 mg) at 120 °C in anhydrous dimethyl sulfoxide (DMSO), followed by acid hydrolysis of the *N*-Boc protecting group. Purification was accomplished by loading the crude reaction mixture to a cartridge (Oasis HLB 3 cc) and eluting with different combinations of solvents. This method removed the chemical impurity while leaving [^{18}F]AV-45 ([^{18}F]**5**) on the cartridge. The final dose was eluted by ethanol. [^{18}F]AV-45 ([^{18}F]**5**) was produced within 51 min (radiochemical yield 42.7 ± 5.9%, decay corrected, n = 3), and the radiochemical purity was >95%. Total chemical impurity per batch (24.1 ± 2.7 μg per batch) was below the limit described in the package insert of Amyvid, florbetapir f18 (chemical mass: less than 50 μg/dose). In summary, [^{18}F]AV-45 ([^{18}F]**5**) was produced efficiently and reproducibly using a cartridge-based SPE purification. This method brings the process closer for routine preparation, similar to the commercially used [^{18}F]FDG.

Key Words: Fluorine-18, solid-phase extraction purification, florbetapir f18, automated radiosynthesis

1. Introduction

Advances in nuclear medicine and biomedical imaging depend on the development of diverse radiopharmaceuticals. Production of radiopharmaceuticals requires a strategy that optimizes a combination of factors, including the radionuclide half-life, structural characteristics of the final molecule or its precursor(s), radiolabeling procedures, and their clinical *in vivo* application.[1] As a logical evolution of improving the preparation of ^{18}F-radiopharmaceuticals in routine production for clinical application, several obstacles need to be resolved. First, the initial radiochemical yield via direct fluorination reaction catalyzed by crown ethers needs to be optimized. Second, based on the reaction kinetics, the reaction time and temperature need to be adjusted to maximize the production of the desired product and to reduce the amount of side products. Third, the final product needs to be simply and readily separated. The separation needs to reduce or eliminate chemical and radiochemical impurities, which is usually accomplished via high-performance liquid chromatography (HPLC) purification. HPLC offers a generally applicable purification method for producing PET radiotracers. However, this method is relatively time consuming, and the dedicated HPLC equipment requires additional space inside the hot cells and regular maintenance. For routine preparation, clinical doses would be best accomplished by a simple, fast, and readily available solid-phase extraction (SPE) purification. Disposable cartridges or solid-phase columns are used only once and conveniently disposed after completion of the purification procedure. This progression is necessary for routine preparation of many radiopharmaceuticals.[2-4] One only needs to examine the history of the development of a method for [^{18}F]FDG preparation, because it is the most widely produced radiopharmaceutical for PET imaging. Previously, in

the 80s and 90s, the production was based on HPLC purification, and the process was time consuming and produced low yield.[5] Improvements reported in the late 80s demonstrated the use of a SN2 fluorination reaction with triflate precursor. The new fluorination of precursor, 1,3,4,6-tetra-O-acetyl-2-O- trifluoromethanesulfonyl-β-D-mannopyranose, enhanced the radiochemical yield and led to significant scaling up of the production from 100 s MBq to 100 s of GBq. After hydrolysis of the acetyl protecting by either acid or base, the final product, [^{18}F]FDG, was "purified" by SPE through different combinations of alumina, C-18, cation exchange, and/or ion retardation. This optimized preparation is now the main production method that is regularly used in commercial or hospital radiopharmacy.[5]

Amyvid ([^{18}F]AV-45, [^{18}F]**5**) was the first amyloid-β PET imaging agent approved by the FDA in 2012. It is now available for routine clinical use in assisting diagnosis of patients suspected of Alzheimer's disease (AD) and also for selecting patients in drug trials.[6-10] Florbetapir f18 PET imaging study is now being used to determine the presence of amyloid load in the brain as a significant risk factor for developing AD. Amyloid-β plaques in the brain are associated with mild cognitive impairment or dementia of uncertain etiology.[11,12] The original method for preparing [^{18}F]AV-45 ([^{18}F]**5**) was based on the final HPLC purification.[13,14] Similar to the [^{18}F]FDG production described above, we previously reported an effort to streamline the [^{18}F]AV-45 ([^{18}F]**5**) production.[15-17] We wish to develop a routine production method, which would be amendable for radiopharmacy operation. The SPE method would likely help make the routine clinical doses of [^{18}F]AV-45 ([^{18}F]**5**) readily available. This situation is a necessary progression for maturing radiochemistry technology to convert a research tracer from laboratory to routine clinical application.

The recommended dose for Amyvid is 370 MBq (10 mCi), containing a maximum of 50 μg chemical mass in one dose, administered as a single intravenous bolus with a total volume of 10 mL or less. Limited Aβ binding sites exist in the brain. Therefore, the molecular activity (chemical amount of carrier or pseudo-carrier is a key determining factor) would be an important factor for consideration. Other chemical impurities may compete for the same Aβ binding sites in the brain.[15] In our previous work, we suggested the use of the SPE method instead of HPLC purification to prepare [^{18}F]AV-45 ([^{18}F]**5**) operated with a semi-automatic synthesizer (module BNU F-A2).[18] The separation procedure included loading of [^{18}F]AV-45 ([^{18}F]**5**) crude reaction mixture on an Oasis HLB 3 cc cartridge and fractional elution of the cartridge with aqueous solutions containing increasing percentage of ethanol. This procedure produced [^{18}F]AV-45 ([^{18}F]**5**) with excellent radiochemical purity (RCP) >95%, in which the "cold" pseudo carrier of AV-136 (**4**, 200–500 μg/batch) remained in the final dose (see Scheme 1).[18] As reported, AV-136, **4** also displayed good binding affinity to Aβ plaques (K_i = 6.37 nM) and might inhibit the binding of [^{18}F]AV-45 ([^{18}F]**5**) (K_i = 2.87 nM) in the human brain to a certain degree.[15-17] Removal or minimizing of this chemical impurity from the final dose formulation is an important goal on meeting the [^{18}F]AV-45 ([^{18}F]**5**) specification.[18]

Based on the previous SPE purification results, additional efforts were made to further reduce the total chemical mass (total amount of chemical impurities) in the final [^{18}F]AV-45 ([^{18}F]**5**) doses. Ultimately, the goal was to maximize the radiochemical yield (RCY) and minimize the chemical impurity to below 50 μg/batch as defined by the package insert of Amyvid. One of the major nonradioactive side products, AV-136, **4**, a pseudo-carrier, was less lipophilic than [^{18}F]AV-45 ([^{18}F]**5**). Thus, it could be better eluted from the reversed-phase

cartridge by manipulating the polarity of the solvent used for eluting the cartridge. Taking advantage of these differences, we have developed a new SPE procedure. In the current study, we reported an optimization of automated radiosynthesis of [^{18}F]AV-45 ([^{18}F]**5**) via an efficient and reliable SPE purification, by which the majority of chemical impurities could be removed from the final doses.

2. Experimental

2.1 General

2.1.1 Reagents and solvents

All reagents and solvents were of analytical reagent grade and purchased from commercial sources (Aldrich, Acros, or Alfa Inc.). They were used without further purification unless otherwise specified.

2.1.2 Instrument and consumable materials

Thin layer chromatography (TLC) was run on pre-coated plates of a silica gel 60 F254 (Merck, Darmstadt, Germany). ^{1}H NMR spectra were obtained on a 600 MHz JMTC-600/54/JJ (Japan Superconductor Technology, Inc., Japan). Chemical shifts are reported as δ values (parts per million) relative to residual protons of deuterated solvent. Coupling constants are reported in Hertz. The multiplicity is defined as singlet (s), doublet (d), triplet (t), broad (br), or multiplet (m). Liquid chromatography–mass spectrometry (LC–MS) analysis was performed on a Triple TOF 5600 (AB SCIEX, Foster City, CA, USA) mass spectrometry with an electrospray ionization (ESI) source, and the LC inlet was Thermo

Scientific UltiMate 3000 chromatographic system. Analytical HPLC was conducted on the Shimadzu LC-20A HPLC system equipped with a multiwavelength UV detector and radiation detector. SPE cartridges (Oasis HLB 3 cc cartridge, Sep-Pak light QMA cartridge) were obtained from Waters (Milford, MA, USA). The [^{18}F]fluoride ion was provided by the Peking University Cancer Hospital.

2.1.3 Liquid chromatography

LC was performed on Thermo Scientific UltiMate 3000 chromatographic system with an autosampler and a column oven. A Phenomenex Luna C18 column (4.6 mm × 150 mm, 5 μm) was employed. Chromatographic separation was achieved with gradient elution by using a mobile phase composed of water containing 10 mM ammonium formate (A) and acetonitrile (ACN) (B). A flowrate of 1 mL/min with the following gradients: from 0 min to 2 min, A 95% and B 5%; from 2 min to 5 min, A 95%–30% and B 5%–70%; from 5 min to 10 min, A 30%–1% and B 70%–99%; from 10 min to 15 min, A 1%–95% and B 99%–5%; and from 15 min to 20 min, A 95% and B 5%. The total HPLC run time was 20 min. The injection volume was 10 μL. Additionally, the compound was determined by UV at 350 nm. The product RCP was confirmed by analytical HPLC (Shimadzu Prominence LC-20AT and SPD-20A, Shimadzu Corp., Japan) connected to a radiation detector (Gabi Star, Raytest Corp., Japan). The analytical HPLC conditions were the same as described above. The RCP of the final product was also measured by Radio-TLC, with a mobile phase ethyl acetate.

2.1.4 Mass spectrometry

The description of LC-MS instrument was included in Section 2.1.2. The ion spray voltage floating was 5500 V, and the source temperature was 600 °C. The ion source gas 1 and gas 2 were both 40 psi, and the curtain gas was 25 psi. The compound declustering potential was 100 V, and the collision energy was 10 eV. The dwell time was set at 0.2750 s per transition. All data collected were acquired and processed using Analyst TF1.7 Software with QuanLynx program. A LCT Premier XE mass spectrometer was used to identify and confirm the chemical impurity of the product. The exact mass measurement was carried out in positive V mode. In the full scan mode, data were acquired in the mass range m/z 100–2000, with a scan time of 1.0 s. A lock spray was used for external calibration with a solution of the leucine-enkefaline as reference. Accurate masses were calculated and used to determine the elementary composition of the components with fidelity of 5 ppm.

2.2 Synthesis

The precursor AV-105, **1**, nonradioactive standard AV-45, **5**, and AV-136, **4** were synthesized according to previous reports (Scheme 1).[19,20]

(E)-4-(2-(6-(2-(2-(2-Chloroethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)-N-methylaniline (Cl-derivative) ***2***

(E)-2-(2-(2-((5-(4-(Methylamino)styryl)pyridin-2-yl)oxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (OTs-derivative) ***3***

A mixture of (*E*)-2-(2-(2-((6-(4-((*tert*-butoxycarbonyl)(methyl)amino)styryl)-pyridin-3-yl)oxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (AV-105, **1**) (100 mg, 0.16 mmol) was stirred in

dichloromethane (20 mL) at room temperature, 1 M HCl (0.5 mL) was added, and the reaction mixture was stirred for 3 days. The solvent was evaporated, and the saturated solution of sodium carbonate (20 mL) was added and extracted with ethyl acetate (20 mL × 3) thrice. The organic layers were combined and dried over anhydrous sodium sulfate and filtered. Then, the filtrate was evaporated and purified by a combiflash (silica gel 25 g) (ethyl acetate:petroleum ether = 0% to 50%) to obtain the Cl-derivative **2** (15 mg, yield 25%) as a yellow solid and OTs-derivative **3** (25 mg, yield 29%) as a yellow solid.

Cl-derivative **2**: ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.13 (s, 1H), 7.75 (d, J = 8.7 Hz, 1H), 7.34 (d, J = 7.3 Hz, 2H), 6.89 (d, J = 16.3 Hz, 1H), 6.80 (d, J = 16.4 Hz, 1H), 6.75 (d, J = 8.6 Hz, 1H), 6.61 (d, J = 7.4 Hz, 2H), 4.50–4.45 (m, 2H), 3.86–3.85 (m, 2H), 3.76–3.74 (m, J = 6.0, 2H), 3.73–3.66 (m, 4H), 3.63–3.60 (m, 2H), 2.86 (s, 3H). HRMS (ESI) calculated for $C_{20}H_{26}ClN_2O_3$ $[M+H]^+$, 377.1626; found, 377.1611.

OTs-derivative **3** (OTs-derivative): ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.11 (d, J = 2.1 Hz, 1H), 7.78 (d, J = 8.1 Hz, 2H), 7.71 (dd, J = 8.7, 2.1 Hz, 1H), 7.34 (d, J = 8.3 Hz, 2H), 7.31 (d, J = 8.2 Hz, 2H), 6.92 (d, J = 7.7 Hz, 2H), 6.84 (d, J = 5.2 Hz, 2H), 6.73 (d, J = 8.6 Hz, 1H), 4.46–4.40 (m, 2H), 4.15–4.14 (m, 2H), 3.81–3.79 (m, 2H), 3.69–3.67 (m, 3H), 3.63–3.59 (m, 4H), 2.91 (s, 3H), 2.41 (s, 3H). HRMS (ESI) calculated for $C_{27}H_{33}N_2O_6S$ $[M+H]^+$, 513.2053; found, 513.2018.

*2.3 Radiosynthesis of [^{18}F]AV-45 ([^{18}F]**5**) and the optimization of SPE purification*

Aqueous solution of the [^{18}F]fluoride ion produced by the cyclotron was passed through a Sep-Pak light QMA cartridge. The cartridge was previously activated with 10 mL 1 M $NaHCO_3$ and 10 mL water and dried with N_2. Then, the ^{18}F activity was eluted with 1.1 mL of Kryptofix 2.2.2 (K2.2.2)/K_2CO_3 solution (11 mg K2.2.2 and 2 mg K_2CO_3 in 0.93 mL ACN and 0.17 mL H_2O). The eluent was then evaporated at 110 °C under an N_2 stream. Additionally, the residue was azeotropically dried twice with 1 mL anhydrous ACN.

*2.3.1 SPE purification of [^{18}F]AV-45 ([^{18}F]**5**) by using an Oasis HLB cartridge*

To the dried [^{18}F]KF/K2.2.2, 1 mg precursor (AV-105, **1**) dissolved in 1 mL anhydrous DMSO was added. The mixture was heated at 110 °C for 10 min, followed by hydrolysis with 3 M HCl (1 mL) at 100 °C for 5 min. After cooling down to room temperature, 0.3 M NaOH (10 mL) was added for neutralization. The crude products were evenly divided into two parts and passed through an Oasis cartridge (pretreated with 10 mL EtOH, followed by 10 mL sterile water). The Oasis cartridges were washed with 2 × 10 mL water. One Oasis cartridge was washed with 2 mL different concentrations of acetonitrile/water (ACN/H_2O, 10%–50%). The other was washed with 2 mL different concentrations of ethanol/water (EtOH/H_2O, 10%–70%). The eluent activity and the retained activity on the Oasis cartridge were recorded. The eluent was also analyzed by HPLC.

Further gradient elution was then explored, including the washing conditions of 8 mL 35% ACN/H_2O, 4 mL 40% ACN/H_2O, 2 mL 45% ACN/H_2O, 4 mL 55% EtOH/H_2O, and 4 mL 60% EtOH/H_2O. The pure product was eluted by 1 mL EtOH. The eluent activity and the

retained activity on the Oasis cartridge were recorded. The eluent and product were analyzed by HPLC.

Finally, we investigated the optimum volume of 35% ACN/H_2O as the elution. The Oasis cartridge was washed with increasing volume (4–8 mL) of 35% ACN/H_2O, followed by elution with 1 mL EtOH. The eluent and retained activities on the Oasis cartridge were recorded. The eluent and product were analyzed by HPLC.

2.3.2 Optimization of fluorination temperature and deprotection conditions

To the dried [^{18}F]KF/K2.2.2 (185–370 MBq), 1 mg precursor (AV-105, **1**) dissolved in 1 mL anhydrous DMSO was added. The mixture was heated at 110 °C, 120 °C, 130 °C or 140 °C for 10 min, followed by hydrolysis with 3 M HCl (1 mL) at 100 °C for 5 min. After cooling down to room temperature, 0.3 M NaOH (10 mL) was added to neutralize. The crude product was passed through an Oasis cartridge. The Oasis cartridge was washed with 10 mL water, 6 mL 35% ACN/H_2O, and 20 mL water in turn. The pure product was eluted by 2 mL EtOH.

To the dried [^{18}F]KF/K2.2.2 (185–370 MBq), 1 mg precursor (AV-105, **1**) dissolved in 1 mL anhydrous DMSO was added. The mixture was heated at 120 °C for 10 min, followed by hydrolysis with 2 M H_2SO_4 (1 mL) at 100 °C for 5 min. After cooling down to room temperature, 0.4 M NaOH (10 mL) was added for neutralization. The purification method was the same as that described above.

*2.4 Automated radiosynthesis of [^{18}F]AV-45 ([^{18}F]**5**) with SPE purification*

The fully automated synthesis of [^{18}F]AV-45 ([^{18}F]**5**) was performed using a BIBD-F synthesizer (Figure 1). The device was built with flexibility in mind and can implement various synthetic procedures. The sequence program of the whole system included the main synthesis unit, purification unit, and formulation unit. Before starting the synthesis, the reagents for the production of [^{18}F]AV-45 ([^{18}F]**5**) were stored in the reagent vials, as follows: (P01) 11 mg K2.2.2 and 2 mg K_2CO_3 in 1.1 mL (0.93 mL ACN and 0.17 mL H_2O) solution; (P02, P03) 1 mL anhydrous ACN; (P04) 1 mg (AV-105, **1**) dissolved in 1 mL anhydrous DMSO; (P05) 1 mL 2 M H_2SO_4 solution; (P06) 10 mL 0.4 M NaOH solution; (P07) 10 mL H_2O; (P08) 6 mL 35% ACN/H_2O; (P09) 20 mL H_2O; and (P10) 2 mL EtOH. The automated synthesis module (Figure 1) was operated in the following sequence. The [^{18}F]fluoride ion (1.85–3.70 GBq) was trapped on a Sep-Pak Light QMA cartridge. Then, the ^{18}F activity was eluted with 1.1 mL eluent (P01) into the reaction vessel. The mixture solution in the reaction vessel was evaporated under a vacuum and N_2 gas flow at 110 °C. The residue was azeotropically dried twice with 1 mL anhydrous ACN (P02, P03) under the same condition. A solution of precursor (1 mg AV-105, **1** in 1 mL anhydrous DMSO, P04) was added to the evaporation residue in the reaction vessel and heated to 120 °C for 10 min. After [^{18}F] fluorination, the reaction mixture was cooled to 90 °C, and 1 mL of 2 M H_2SO_4 acid solution (P05) was added to remove the *N*-Boc group at 100 °C for 5 min. The 10 mL 0.4 M NaOH solution (P06) was added for neutralization, and the mixture was subsequently passed through an Oasis HLB 3 cc cartridge. Subsequently, the cartridge was rinsed with 10 mL deionized water (P07), 6 mL 35% ACN/H_2O (P08), and 20 mL deionized water (P09) in

turn. After drying the cartridge under a N_2 flow, the pure product was eluted with 2 mL EtOH (P10) into the transfer vial containing 7 mL of 0.9% saline solution. The final product was moved to the final product vial containing 11 mL of 0.9% saline solution. The solution was passed through a 0.22 μm sterile filter (Medical Millex-GV; Millipore, Billerica, MA, USA) into a sterile vial to generate the dose for injection.

2.5 Quality control

The chemical impurities and specific activity of [^{18}F]AV-45 ([^{18}F]**5**) were measured and quantified with calibration curves of F-19 standards by HPLC (Figure S1). RCP was measured by radio-HPLC and radio-TLC, and the conditions were as described above (Section 2.1.3). The R_f value of AV-45, **5**, on the TLC plate was 0.7. Residual K2.2.2 was run on pre-coated plates of silica gel 60 F254 with a mobile phase EtOH/30% ammonia (v/v = 9/1). In addition, the spots were stained with iodine vapor to make them visible. The pH of the final product was measured by applying a small volume (20 μL) to a pH-indicator strip (pH range: 0.0–14.0) and compared with the standard color chart provided by the test manufacturer. The residual organic solvents (ACN and DMSO) were quantified using a gas chromatograph system (Agilent 6820 GC, Agilent Technologies, USA) equipped with an FID detector and a DB-FFAP capillary column (30 m × 0.32 mm × 0.5 μm, Agilent Technologies, USA).

3. Results and Discussion

3.1 Chemical synthesis

The synthesis of Cl-derivative **2** and OTs-derivative **3** is illustrated in Scheme 1. Precursor, AV-105, **1**, was hydrolyzed by 1 M HCl to give OTs-derivative **3** in 29% yield. In the same reaction, the Cl-derivative **2** was produced in 25% yield. The "cold" AV-45, **5**, was prepared as described previously.[20] All compounds were characterized by ^{1}H NMR and HRMS. The results were consistent with the proposed structures. The authentically prepared AV-45, **5**, and related derivatives, **2**, **3**, and **4**, were employed as standards for establishing the analytical HPLC methods to measure the residual chemical quantity. The calibration curves are included in the Supporting information.

*3.2 Radiosynthesis of [^{18}F]AV-45 ([^{18}F]**5**) and the optimization of SPE purification*

*3.2.1 SPE purification of [^{18}F]AV-45 ([^{18}F]**5**) by using an Oasis HLB cartridge*

The [^{18}F]AV-45 ([^{18}F]**5**) radiosynthesis was performed according to the reaction shown in Scheme 2. The radiotracer was produced from the *O*-tosylated precursor (AV-105, **1**) via a standard nucleophilic substitution fluorination reaction. After an acid hydrolysis of the *N*-Boc protection group, the crude reaction mixture was subjected to SPE purification.

Many recent reports describe the SPE purification method for different PET radiotracers. This technique has been successfully applied to prepare a number of clinically relevant radioactive tracers, including [^{18}F]FDG,[4,21] [^{18}F]FMISO,[22] [^{11}C]PiB,[23] [^{18}F]FMZ[2], and [^{18}F]FES.[24] A SPE purification method for preparing [^{18}F]AV-45 ([^{18}F]**5**) had been previously reported without HPLC purification.[18] However, the reported SPE purification

method did not fully remove the hydroxyl derivative, AV-136, **4**, which could potentially interfere with the detection of Aβ plaques in the brain. Due to its relatively high binding affinity (K_i = 6.37 ± 3.75 nM),[15] AV-136, **4**, might compete for the same Aβ binding sites in the brain.

Since the hydroxyl derivative, AV-136, **4** is more hydrophilic than [^{18}F]AV-45 ([^{18}F]**5**), we employed the following separation steps: 1) the crude reaction mixture was loaded on an Oasis HLB 3 cc cartridge; and 2) the Oasis HLB 3 cc cartridge was fractionally eluted by aqueous solutions with increasing ethanol or acetonitrile content. In addition, LC–MS technique and calibration curves of standards with HPLC were used to identify and quantify the residual chemical impurities in the SPE-purified [^{18}F]AV-45 ([^{18}F]**5**) product. Using the optimized synthesis, the [^{18}F]AV-45 ([^{18}F]**5**) synthesis was finally implemented on an automated synthesis module (BIBD-F).

Commercially available Oasis HLB 3 cc cartridge is a reversed-phase SPE cartridge packed with polymer-based sorbent, which was selected for this study. Compared with the relatively hydrophilic AV-136, **4**, the desired [^{18}F]AV-45 ([^{18}F]**5**) was not washed out by the selected eluent but rather retained on the cartridge. To facilitate purification, we used different combinations of EtOH/H_2O or ACN/H_2O to elute the SPE cartridge and remove the side product, AV-136, **4**. This study aimed to maximize the removal of the impurity, AV-136, **4**, while retaining the desired product, [^{18}F]AV-45 ([^{18}F]**5**), on the cartridge. Afterward, the desired product, [^{18}F]AV-45 ([^{18}F]**5**), was left on this Oasis cartridge and finally eluted by ethanol.

To test the efficiency of AV-136, **4**, removal from the cartridge, various combinations of eluent were tested. As shown in Figure 2A, when the Oasis cartridge was eluted with 2 mL of increasing ACN/H_2O concentrations of 10%, 20%, 30%, 40%, and 50%, the removal of impurity AV-136, **4**, and product loss of [^{18}F]AV-45 ([^{18}F]**5**) were measured and calculated. When ACN/H_2O at 10% to 30% was selected as the eluent for rinsing the cartridge, the removal of impurity AV-136, **4**, was <10%. Meanwhile, the desired product, [^{18}F]AV-45 ([^{18}F]**5**), still remained on the cartridge (the loss was <5%). When the ACN content was increased to 40% in the eluent, more than 80% of AV-136, **4** was removed, and the loss of product, [^{18}F]AV-45 ([^{18}F]**5**), was <10%. Moreover, as expected, the use of 50% ACN/water as the eluent resulted in a loss of 70% of the product. Using 30%–40% ACN/H_2O as the eluent might remove most of the undesired impurity, AV-136, **4**.

When the Oasis cartridge was eluted with 2 mL of either 10%, 20%, 30%, 40%, 50%, 60%, or 70% EtOH/H_2O, the removal of impurity AV-136, **4** and loss of product were calculated (Figure 2B). When the cartridge was eluted with either 10% to 50% EtOH/H_2O, the removal of impurity AV-136, **4** and the loss of product, [^{18}F]AV-45 ([^{18}F]**5**) were very low. When the EtOH fraction was increased to 60%, the AV-136, **4** removal was approximately 50%, and less than 10% of the product [^{18}F]AV-45 ([^{18}F]**5**) was lost. When the EtOH fraction was increased to 70%, 21% of the product [^{18}F]AV-45 ([^{18}F]**5**) was lost.

To further optimize the SPE purification, we explored the combinations of a narrow range of EtOH/H_2O and ACN/H_2O as the eluting solvent. When 8 mL of 35% ACN/H_2O was used to wash the Oasis cartridge, close to 100% of AV-136, **4**, was removed, and close to 89% of the desired product was retained on the cartridge (Figure 2C). When the volume of

35% ACN/H_2O was adjusted, 6 mL eluent afforded the best results (Figure 2D), i.e., more than 90% of impurity was removed, and >90% of the product remained on the cartridge. With this elution condition for SPE purification, eluting the Oasis HLB 3 cc cartridge with 6 mL of 35% ACN/H_2O resulted in the removal of approximately 95% of AV-136, **4**, and more than 90% of the desired product, [^{18}F]AV-45 ([^{18}F]**5**), was retained on the cartridge. The final doses of [^{18}F]AV-45 ([^{18}F]**5**) on the Oasis cartridge were completely eluted by ethanol, thereby resulting in a 100% pure product.

3.2.2 Optimization of fluorination temperature and deprotection conditions

Two other main chemical impurities, compounds **2** and **3**, were found in the SPE-purified product. The fluorination reaction most likely produced the Cl-derivative **2** and the OTs-derivative, **3**, (Scheme 1), which were confirmed by testing the crude reaction mixture by a high-resolution LCT Premier XE mass spectrometer. The protonated molecular mass of impurity A (theoretical 377.1626 Da, measured 377.1631 Da) showed a 1.2 ppm error. In addition, the molecular formula was $C_{20}H_{26}ClN_2O_4$, which was consistent with the Cl-derivative **2**. The protonated molecular mass of impurity B (theoretical 513.2053 Da, measured 513.2047 Da) showed a 1.5 ppm error, and the molecular formula was $C_{27}H_{33}N_2O_6S$. This result was consistent with the OTs-derivative **3**. The corresponding Cl- and OTs-derivatives, **2** and **3**, were authentically synthesized, and the concentrations of **2** and **3** in the final formulated solution were calculated by the calibration curves of standards with HPLC. The conditions are described in Section 2.1.3. The calibration curves of Cl-derivative **2**, OTs-derivative **3**, AV-136, **4**, and AV-45, **5**, by using authentically synthesized samples are shown in the Supporting Information (Figure S1).

To minimize the residual amount of OTs-derivative **3**, fluorination reaction was carried out for 10 min at 110 °C, 120 °C, 130 °C, or 140 °C. Various reaction temperatures were tested to determine their influence on the residual amount of OTs-derivative **3**, synthesis time, the total mass of impurities, and the RCYs (decay corrected). The results are summarized in Table 1. When the fluorination reaction temperature was increased to 110 °C–140 °C, comparable decay corrected RCYs of [^{18}F]AV-45 ([^{18}F]**5**), remained mass of AV-136, **4** (13–16 μg per batch) and Cl-derivative **2** (14–18 μg per batch) in the final formulated solution were observed, while the total mass of OTs-derivative, **3**, was significantly reduced. When the fluorination reaction was carried out at ≥130 °C, no OTs-derivative, **3**, was found in the reaction mixture. Considering that the synthesis module was not made to sustain the temperature above 120 °C, we selected 120 °C as the fluorination reaction temperature, at which 1–2 μg of OTs-derivative **3**, remained in the final dose.

As speculated, the impurity Cl-derivative **2**, might be produced from the 3 M HCl acid solution. To eliminate the production of Cl-derivative **2**, which was possibly caused by using HCl, 2 M H_2SO_4 was used instead for the acid hydrolysis step to remove the *N*-Boc protection group. The amount of Cl-derivative **2** was significantly reduced to 1–2 μg per batch by using 2 M H_2SO_4 (Table 2). No significant difference was observed in the total mass of AV-136, **4** (13–17 μg per batch) and the OTs-derivative **3** (1–3 μg per batch) in the final formulated product. It is worth mentioning that, in theory, when H_2SO_4 was used for acid hydrolysis, without introducing chloride ion, the final formulated solution should not contain any Cl-derivative **2**. However, the experimental results showed otherwise. Traces of Cl-derivative **2** (1–2 μg) were still observed in the final product (Table 2). It is likely that the

Cl sources could have been derived from either during the synthesis of *O*-tosylated precursor, **1**, or from the [^{18}F]fluoride ion solution derived from cyclotron. The amount of Cl-derivative **2** in the final formulation of [^{18}F]AV-45 ([^{18}F]**5**) was very low (less than 5 μg per batch). At this low level, it might not play an important role in inhibiting the binding of [^{18}F]AV-45 ([^{18}F]**5**) in the brain.

The optimized procedure for preparing [^{18}F]AV-45 ([^{18}F]**5**) was established as follows. [^{18}F]fluoride (1.85–3.70 GBq) was eluted from a QMA cartridge with the K2.2.2/K_2CO_3 solution. The solution was azeotropically dried twice with 1 mL anhydrous ACN under N_2 gas. The *O*-tosylated precursor, **1**, (1 mg in 1 mL of anhydrous DMSO) was added to this reaction vessel containing the activated [^{18}F]fluoride. The reaction mixture was heated to 120 °C for 10 min. After the fluorination reaction, the reaction mixture was cooled to 90 °C, and 1 mL of 2 M H_2SO_4 acid solution was added to remove the *N*-Boc group at 100 °C for 5 min. NaOH at 10 mL of 0.4 M solution was added for neutralization, and the crude reaction mixture was subsequently loaded on an Oasis HLB 3 cc cartridge. The cartridge was sequentially rinsed with 10 mL of deionized water first and then 6 mL 35% ACN/H_2O and 20 mL deionized water. After drying the cartridge under N_2, the pure product was eluted by 2 mL of EtOH into a vial containing 7 mL of 0.9% saline solution. The solution was transferred to the final product vial containing 11 mL of 0.9% saline solution (a total of 20 mL volume). The final [^{18}F]AV-45 ([^{18}F]**5**) product solution was tested for quality control and then released (Table 3).

*3.3 Radiosynthesis of [^{18}F]AV-45 ([^{18}F]**5**) with SPE purification on an automated synthesizer*

The radiosynthesis for [^{18}F]AV-45 ([^{18}F]**5**) was tested further by using an automated synthesizer (BIBD-F, Figure 1). The radioactivity for the preparation was increased ($^{18}F^-$ activity ranging from 1.85 GBq to 3.70 GBq). The RCY of [^{18}F]AV-45 ([^{18}F]**5**) was 42.7 ± 5.9% (decay corrected, n = 3), and the total synthesis time was 51 ± 2 min. Radio-HPLC and radio-TLC analysis showed that the RCP of [^{18}F]AV-45 ([^{18}F]**5**) was >95%. The specific activity at the end of synthesis was 254 ± 99 GBq/mg. The radiochemical identity was confirmed by analytical HPLC with the nonradioactive standard (spike), AV-45, **5**, which showed identical retention time under the HPLC conditions described in the Methods section (Figure 3). All of the necessary quality control steps for release criteria, including physical characteristics, pH value, residual K2.2.2, residual acetonitrile, and DMSO of the solution met the drug release requirements (Table 3). The total mass of chemical impurities in the product was calculated (24.1 ± 2.7 μg per batch). In addition, the impurity concentration in the final formulation was lower than 3 μg/mL in a total of 20 mL volume. Chemical mass dose per injection met the purity requirement, i.e., the total amount of chemical was lower than 50 μg as defined in the package insert of Amyvid ([^{18}F]AV-45, [^{18}F]**5**).

4. Conclusions

An improved process for preparing Amyvid ([^{18}F]AV-45, [^{18}F]**5**) by using a SPE purification method was developed. The desired product was prepared with a high radiochemical yield (42.7 ± 5.9%, decay corrected) and high radiochemical purity (>95%) in 51 min. Specifically, the total chemical mass in the final dose met the release criteria (total

chemical lower than 50 μg/dose). This modified radiosynthesis might be suitable for routine preparation for clinical application.

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Table 1 Optimization of fluorination reaction temperature (n = 3)

Fluorination temperature (°C)	RCY* of [^{18}F]AV-45 (%)	Total time (min)	Mass of AV-136, **4** (μg)**	Mass of Cl-derivative **2** (μg)**	**Mass of OTs-derivative 3 (μg)****
110	34.2 ± 2.0	45 ± 3	13.2 ± 1.25	17.7 ± 6.34	**21.7 ± 10.6**
120	30.2 ± 2.0	49 ± 6	14.3 ± 1.78	17.2 ± 6.61	**1.32 ± 2.28**
130	27.5 ± 1.8	54 ± 2	16.5 ± 0.75	14.1 ± 3.15	**0**
140	30.5 ± 3.7	55 ± 4	14.5 ± 2.48	14.1 ± 1.25	**0**

*Decay corrected;

**Total mass per batch, amounts of chemical impurities were calculated based on calibration curves (supporting Figure S1)

Table 2 Optimization of acid hydrolysis (n = 3)

Acid for Hydrolysis	RCY* of [^{18}F]AV-45 (%)	Total time (min)	Mass of AV-136, **4** (μg)**	**Mass of Cl-derivative 2 (μg)****	Mass of OTs-derivative **3** (μg)**
2 M H_2SO_4	34.2 ± 4.8	50 ± 3	17.2 ± 3.71	**2.46 ± 1.35**	3.44 ± 1.98
3 M HCl	30.2 ± 2.0	49 ± 6	14.3 ± 1.78	**17.2 ± 6.61**	1.32 ± 2.28

*Decay corrected;

**Total mass per batch, amounts of chemical impurities were calculated based on calibration curves (supporting Figure S1)

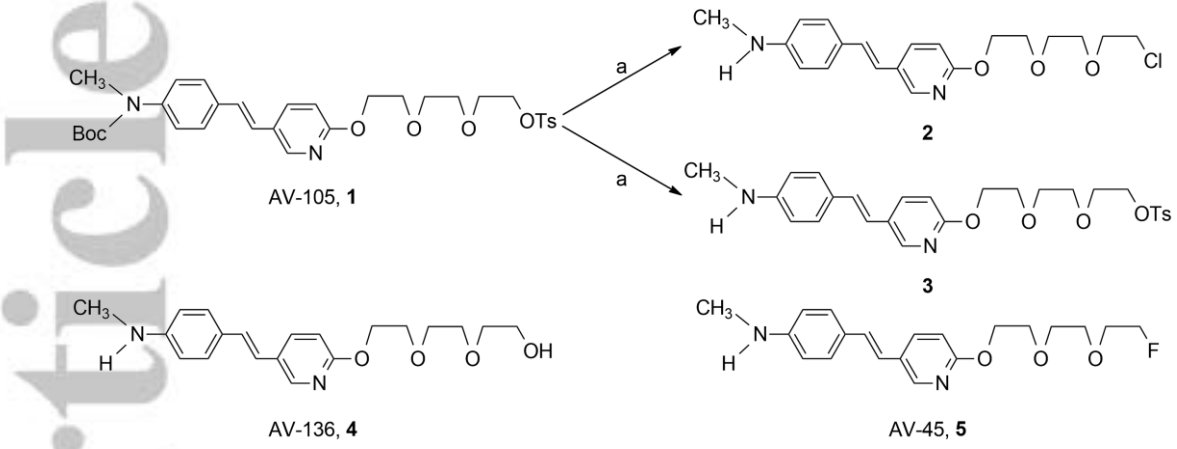
Table 3 QC tests, specifications and results from three validation batches of [^{18}F]AV-45 ([^{18}F]**5**) (n = 3)

Parameter	Value	Parameter	Value
Synthesis time	51 ± 2 min	Radiochemical purity	96.1 ± 0.5%
Radiochemical yield (EOS) *	42.7 ± 5.9%	Radionuclide identity	^{18}F ($t_{1/2}$ = 111.9 ± 1.7 min)
Physical characteristics	Clear, colorless liquid; no suspended particles	Residual DMSO	<0.5%
pH value	6–7	Residual acetonitrile	<0.041%
Activity concentration (EOS)	34.5 ± 3.6 MBq/mL	Content of K2.2.2	<50 μg/mL
Specific activity (EOS)**	254 ± 99 MBq/μg	Chemical purity**	1.21 ± 0.13 μg/mL

*Decay corrected

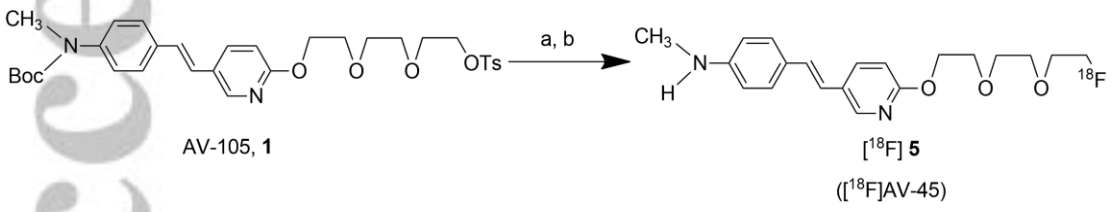
**Amounts of chemical impurities and the specific activity were calculated based on calibration curves (supporting Figure S1)

Scheme 1 Synthesis of Cl-derivative **2** and OTs-derivative **3**.



Reagents and conditions: (a) 1 M HCl, DCM, rt, 3 d

Scheme 2 Radiosynthesis of [^{18}F]AV45 ([^{18}F]**5**).



Reagents and conditions: (a) [^{18}F]KF/K2.2.2, K_2CO_3, DMSO, 110–140 °C, 10 min; (b) 3 M HCl (or 2 M H_2SO_4), 100 °C, 5 min.

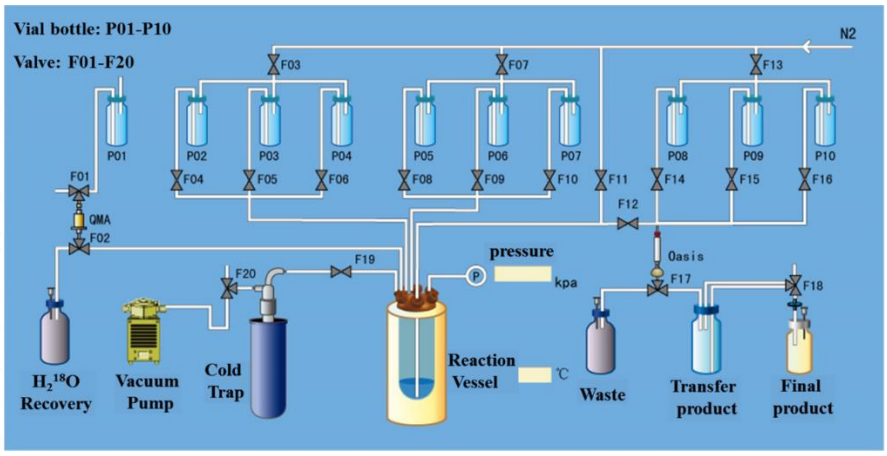


FIGURE 1 Module diagram (BIBD-F) of automated radiosynthesis of [^{18}F]AV-45 ([^{18}F]**5**).

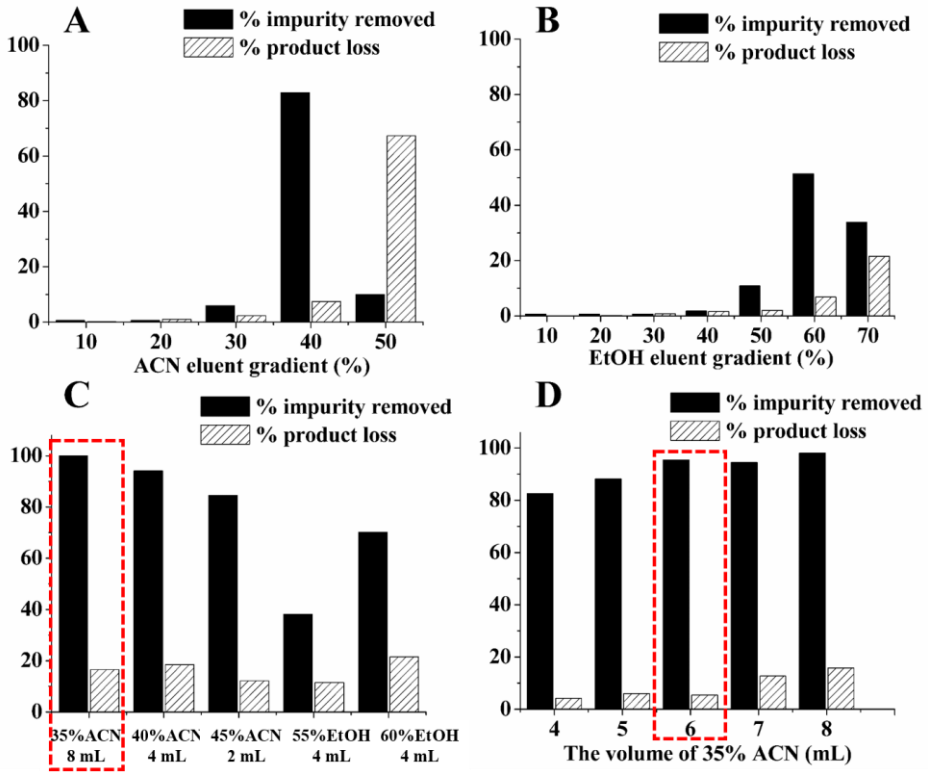


FIGURE 2 After loading the crude reaction mixture on to a solid-phase cartridge (Oasis HLB 3 cc), it was eluted with different solvents. The results of a series of eluents with different solvent combinations for solid-phase extraction (SPE): (A) Eluates: 2 mL of 10%–50% ACN/H_2O; (B) Eluates: 2 mL of 10%–70% EtOH/H_2O; (C) Different ratio of ACN/H_2O or EtOH/H_2O; (D) different volume of 35% ACN/H_2O. The optimal combinations of eluent, maximizing removal of chemical impurity (AV136, **4**) in black and retaining the desired product, [^{18}F]AV45 ([^{18}F]**5**) in stripe, were indicated by red dotted frame.

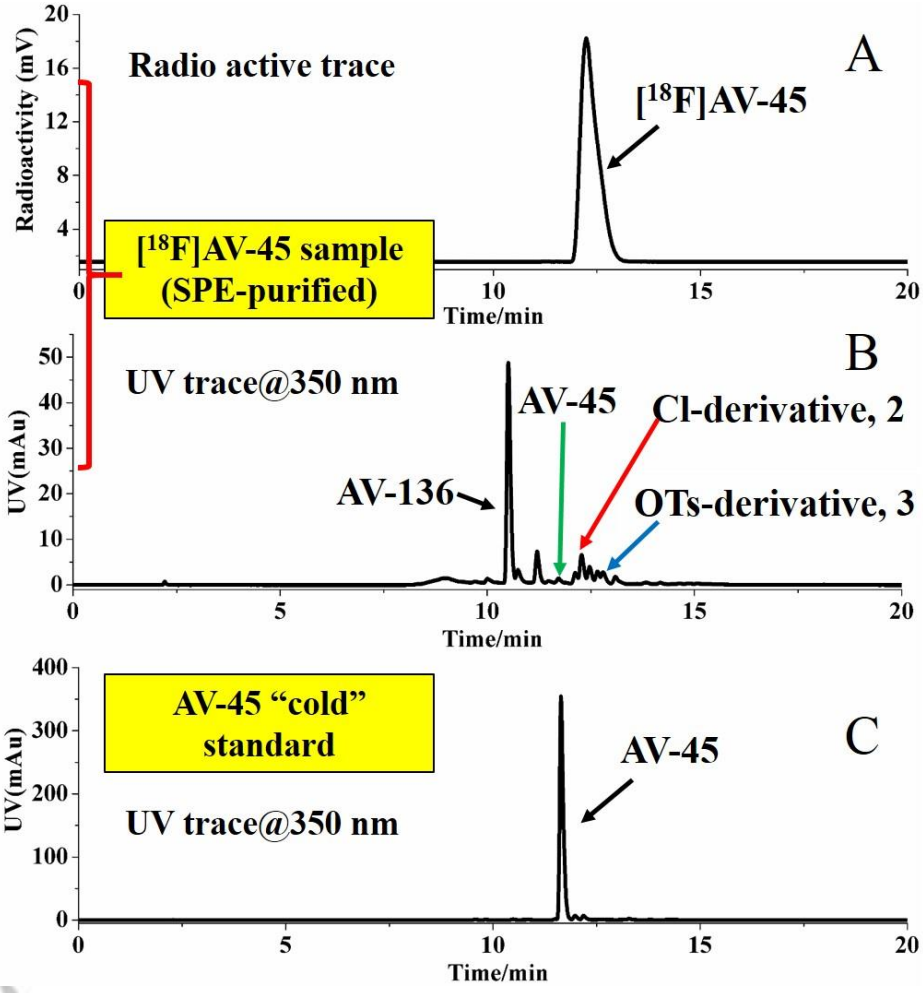


FIGURE 3 Analytical HPLC profile of SPE-purified [^{18}F]AV-45 ([^{18}F]**5**) sample (undiluted into a formulation): trace A was the radio trace of [^{18}F]AV-45 ([^{18}F]**5**, t_R = 12.2 min); trace B was the UV trace of sample (@350 nm), the black arrow represent AV-136 (**4**, t_R = 10.5 min), the green arrow represent AV-45 (**5**, t_R = 11.7 min), the red arrow represent Cl-derivative (**2**, t_R = 12.3 min), and the blue arrow represent OTs-derivative (**3**, t_R = 12.8 min). Analytical HPLC profile of "cold" standard AV-45, **5**: trace C was the UV trace of AV-45 (**5**, t_R = 11.6 min, @350 nm). HPLC was acquired using a Phenomenex Luna C18 column (4.6 mm × 150 mm, 5 μm) and UV spectroscopic detection at 350 nm with a flow rate of 1 mL/min and the following gradient: from 0 min to 2 min, A 95% and B 5%; from 2 min to 5 min, A 95%–30% and B 5%–70%; from 5 min to 10 min, A 30%–1% and B 70%–99%; from 10 min to 15 min, A 1%–95% and B 99%–5%; and from 15 min to 20 min, A 95% and B 5%. Mobile phase A: water with 10 mM ammonium formate buffer and mobile phase B: ACN. The total HPLC run time was 20 min. The injection volume was 10 μL.