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*Original Article*

# Automated production of [$^{18}$F]Flortaucipir for PET imaging of tauopathies

Huailei Jiang[1,2], Manoj K Jain[1], Hancheng Cai[1]

[1]*Department of Radiology, Mayo Clinic, Jacksonville, FL, USA;* [2]*Karmanos Cancer Institute, Detroit, MI, USA*

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**Abstract:** Radiotracer [$^{18}$F]Flortaucipir is an FDA-approved diagnostic agent for PET imaging of density and distribution of abnormal tau protein deposition (tauopathies) in Alzheimer's disease. A high-yield automated method for routine GMP-compliant [$^{18}$F]Flortaucipir production is desired to meet increasing clinical need. In this work, we reported an automated radiosynthesis of [$^{18}$F]Flortaucipir in a RNplus Research module and the quality control (QC) tests for human use under full GMP compliance. Briefly, automated radiosynthesis of [$^{18}$F]Flortaucipir was processed via nucleophilic radiofluorination of precursor AV1622 and followed by acid hydrolysis in a RNplus Research module, which included the radiosynthesis, semi-preparative high-performance liquid chromatography (HPLC) purification, and the final formulation via solid phase extraction (SPE). The final products were obtained in non-decay corrected radiochemical yields of 14.8-16.6% (n = 3) within total synthesis time of 55 min. The radiochemical purities of [$^{18}$F]Flortaucipir were > 99.9% and the molar activities were 247.9-384.8 GBq/μmol at end of synthesis. The results of QC tests met all the specifications for human use. In conclusion, [$^{18}$F]Flortaucipir was reproducibly achieved with desired radiochemical yield and high radiochemical purity and molar activity. Three GMP compliant validation runs and QC results demonstrated the efficacy of this method for automated production of [$^{18}$F]Flortaucipir for human use.

**Keywords:** PET, Tau, [$^{18}$F]Flortaucipir, radiosynthesis, quality control

## Introduction

Tau protein aggregation as neurofibrillary tangles (NFT) is one of the most well-established pathological hallmarks of Alzheimer's disease (AD) [1, 2]. The build-up of Tau protein (tauopathies) has also been found in the brains of patients with other neurodegenerative diseases, such as frontotemporal dementia (FTD), dementia with Lewy bodies (DLB), atypical parkinsonian syndromes like progressive supranuclear palsy (PSP), and corticobasal syndrome (CBS) [3, 4]. With specific radiotracer, positron emission tomography (PET) imaging allows for noninvasive characterization and quantification of tau protein aggregation in human brain [4-8]. In vivo detection and quantification of aggregated Tau density and distribution by PET has the potential to advance precision diagnosis and monitor the efficacy of novel anti-Tau therapy longitudinally for various neurodegenerative diseases [4, 6]. [$^{18}$F]Flortaucipir, also called [$^{18}$F]AV1451 and originally [$^{18}$F]T807, is a well-characterized PET radiotracer that specifically binds to the tau protein in human brain [6, 9-11]. Flortaucipir F 18 Injection ([$^{18}$F]Flortaucipir) was approved by US FDA in 2020 with the brand name of TAUVID for imaging patients with AD [12]. As FDA-approved the first diagnostic agent for Tau PET imaging, it is also expected to noninvasively assess the build-up and distribution of tauopathies in other neurodegenerative diseases.

Several PET radiochemistry groups have reported the radiosynthesis of [$^{18}$F]Flortaucipir via conventional nucleophilic radiofluorination and following with/without acid hydrolysis in different automated radiosynthesiziers or modules in the past ten years [10, 11, 13-19]. In the initial reported methods, the precursor with a nitro leaving group on the pyridine (nitro-precursor, T807P), or its N-Boc-protected nitro-precursor (N-Boc-T807P) was employed for the nucleophilic radiofluorination. Although the radiolabeling yields were acceptable, it was difficult for

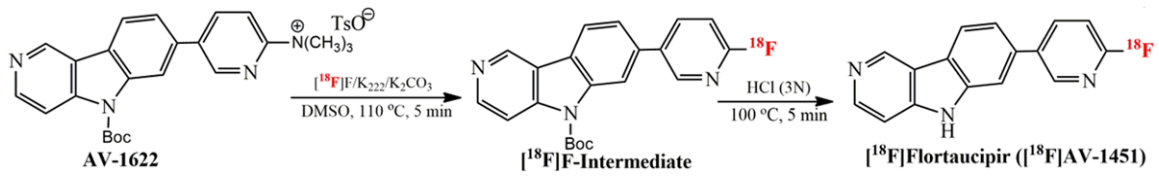


**Figure 1.** Reaction scheme for radiosynthesis of [18F]Flortaucipir.

the separation between the nitro-precursor and [18F]Flortaucipir product, resulting in complicated HPLC purification process or additional metal reduction step for better separation. Later, Xiong et al. developed a facile radiosynthesis with a new precursor using trimethylammonium as leaving group (known as AV1622), which significantly improved the HPLC purification process, such as removing the use of metal reduction [20]. Followed by the radiofluorination, the deprotection of radiolabeled intermediate was undertaken with either addition of diluted HCl or one-pot heating. These radiosynthesis methods have been successfully applied on GE TRACERlab FX F-N module [13, 14, 16, 17, 20], microwave radiosynthesis module [19], and Raytest® synthesis module [18]. However, these reported methods are various, and the two-step methods generally require a large volume reaction vessel for the radiolabeling with precursor AV1622 and following hydrolysis and purification, which limited its use for modules with smaller reaction vessels.

To implement the radiosynthesis of [18F]Flortaucipir with precursor AV1622 in our PET radiochemistry laboratory for human use and expand this automated radiosynthesis to more radiosynthesizers or modules with different sizes of reaction vessels, we modified the reported methods by decreasing the solvents/agents used in the radiolabeling and deprotection [18-20]. Specifically, we reported an updated radiosynthesis of [18F]Flortaucipir with AV1622 precursor (**Figure 1**) on a Synthra RNplus Research module, and conducted full quality control tests under GMP compliance.

## Materials and methods

### *Materials and equipment*

Unless otherwise stated, reagents, solvents, and chemicals were purchased from commercially available vendors and used without further purification. The [18F]Flortaucipir precursor AV1622 and reference standard AV1451 were provided by Avid Radiopharmaceuticals (Philadelphia, PA, USA). Ethanol (EtOH), acetonitrile (MeCN), dimethyl sulfoxide (DMSO), potassium carbonate ($K_2CO_3$), ammonium acetate, and Millex-GV Syringe Filter Unit (0.22 μm, PVDF, 33 mm, gamma sterilized) were purchased from Millipore Sigma (St. Louis, MO, USA). Kryptofix ($K_{2.2.2}$) was purchased from ABX (Radeberg, Germany). Hydroxyl chloride 3 N (HCl) solution was purchased from RICCA Chemical (Arlington, TX, USA). Sodium hydroxide 0.5 N (NaOH) was purchased from Aqua Solutions (Deer Park, TX, USA). QMA Carbonate Plus Light Cartridge (46 mg Sorbent per Cartridge, 40 μm), Oasis HLB Plus Short Cartridge (225 mg Sorbent per Cartridge, 60 μm) and tC18 Plus Short Cartridge (400 mg Sorbent per Cartridge, 37-55 μm) were purchased from Waters (Milford, MA, USA). Both 0.9% Sodium chloride for injection, USP and sterile water for injection, USP were purchased from Hospira (Lake Forest, IL, USA). Fresh deionized water (18.2 MΩ•cm at 25°C) was generated from Milli-Q Direct Water Purification System (Millipore Sigma, Billerica, MA, USA), and used for all standard and eluent solutions preparation. The $K_{2.2.2}/K_2CO_3$ stock solution for [18F]fluoride elution was formulated with 240 mg $K_{2.2.2}$ and 40 mg $K_2CO_3$ in 19.4 mL acetonitrile and 0.6 mL Millipore water, and passed through a Millex-GV filter (Millipore Sigma, St. Louis, MO, USA). Prior to use, QMA cartridge was conditioned with 5 mL deionized water, and HLB and tC18 cartridges were conditioned with 5 mL ethanol, and then 5 mL deionized water.

Automated radiosynthesis of [18F]Flortaucipir was carried out in a RNplus Research module, which included radiotracer separation by a built-in semi-preparative high-performance liquid chromatography (HPLC) system and ultraviolet (UV)/radioactivity detectors. The [18F]Flortaucipir purification was conducted on a semi-preparative HPLC column (ZORBAX Eclipse XDB

80 Å C18, 5 μm, 9.4 × 250 mm) at the UV wavelength of 270 nm. The HPLC loop volume was 3 mL and the mobile phase was a mixture of acetonitrile and 10 mM ammonium acetate (v/v = 30/70) with a flow rate of 4 mL/min. Radioactivity was determined with a Capintec® CRC 55tPET dose calibrator (Capintec, Inc., Florham Park, NJ, USA). Ultimate 3000 HPLC (Thermo Scientific, Waltham, MA, USA) equipped with ultra violet (UV) and radioactivity detectors (Eckert & Ziegler, Wilmington, MA, USA) was used to determine radiochemical purities and identities, and chemical impurities on an analytical reverse-phase column (Phenomenex Luna C18(2) 100 Å, 5 μm, 4.6 × 250 mm) at the UV wavelength of 270 nm. The sample injection volume was 20 μL and the mobile phase was a mixture of acetonitrile and 10 mM ammonium acetate (v/v = 40/60) with a flow rate of 1 mL/min. Radio thin layer chromatography (Radio-TLC) was applied with BioScan AR-2000 (Eckert & Ziegler, Wilmington, MA, USA) to confirm the radiochemical purity during production validation process. The Radio-TLC mobile phase was a mixture of diethyl ether/methanol/28-30% ammonium hydroxide (v/v/v = 9:1:0.1). The [18F]Flortaucipir product was diluted with acetone (v/v = 1:100) and then spotted (1-2 μL) at the original of an neutral aluminum oxide 60 $F_{254}$ TLC strip (Millipore Sigma, St. Louis, MO, USA). Residual solvents were analyzed by Tracer 1310 gas chromatograph (GC) (Thermo Scientific, Waltham, MA, USA) with a TG-WAXMS column (30 m × 0.53 mm × 0.50 μm). The injection volume was 0.5 μL. The GC method was holding at 35°C for 2 min after injection, increasing to 80°C with a rate of 20°C/min, and then reaching to 140°C at a rate of 40°C/min.

*RNplus research module configuration*

RNplus research module (Synthra, Hamburg, Germany) is a computer controlled and user programmable synthesizer for completely automated multi-step productions of various F-18 radiotracers. The setup for [18F]Flortaucipir production in RNplus research module was detailed in **Figure 2**. In the optimized module configuration, vials A1-A6 and reactor 1 were used for [18F]Flortaucipir radiosynthesis followed by HPLC purification on column-1, and then formulated via solid phase extraction (SPE) from vials C1-C3. Vials S1-S3 were used for system self-cleaning. Vials B1-B6, reactor 2 and HPLC column-2 were not required for [18F]Flortaucipir production.

*Radiosynthesis, purification, and formulation of [18F]Flortaucipir*

Radioactive [18F]Fluoride was produced via the $^{18}O(p, n)^{18}F$ nuclear reaction with a GE PETtrace 860 cyclotron, and delivered directly into a Synthra RNplus Research module, where it was trapped on an anion exchange QMA cartridge. Elution of [18F]fluoride with a mixture solution (1 mL) of $K_{2.2.2}/K_2CO_3$ into reaction vial was followed by azeotropic drying under helium flow and vacuum at 70°C for 3.0 min, then at 100°C for 2.0 min. A solution of the precursor AV1622 (1 mg) in anhydrous DMSO (1 mL) was added to the reaction vial and radiofluorination of AV1622 was then achieved by heating at 110°C for 5 minutes, followed by de-protection using 3 N HCl (aq, 0.5 mL) at 100°C for 5 minutes. After cooling to 50°C, the crude [18F]Flortaucipir solution was neutralized with 0.5 N NaOH (aq, 3.5 mL). The resulting mixture was passed through an Oasis HLB cartridge. The retained crude [18F]Flortaucipir was washed with 5 mL of water, and then eluted off the Oasis HLB cartridge using 1.5 mL of acetonitrile. The crude [18F]Flortaucipir was diluted with 1.5 mL of water and then loaded onto a semi-preparative C18 HPLC column for purification (**Figure 3**) using the isocratic elution (70% 10 mM ammonium acetate: 30% acetonitrile) at a flow rate of 4 mL/minute. The HPLC fraction containing the purified [18F]Flortaucipir ($t_R$ = 14-15 min) was collected and diluted with 30 mL of water, and then passed through a tC18 cartridge. The retained [18F]Flortaucipir was washed with 10 mL of sterile water, and then eluted off the tC18 cartridge with 1.2 mL of dehydrated ethanol and formulated with 10 mL of 0.9% sodium chloride injection, USP. The final formulated drug product [18F]Flortaucipir was delivered to the 30 mL vial through a sterilizing 0.22 μm filter in an ISO 5 dispensing hot cell.

*Quality control and stability test of [18F]Flortaucipir*

After the radiosynthesis of [18F]Flortaucipir, the sample was withdrawn from the final product vial for the quality control tests, which included appearance, pH, half-life, radiochemical purity and identity, chemical impurities, residual sol-

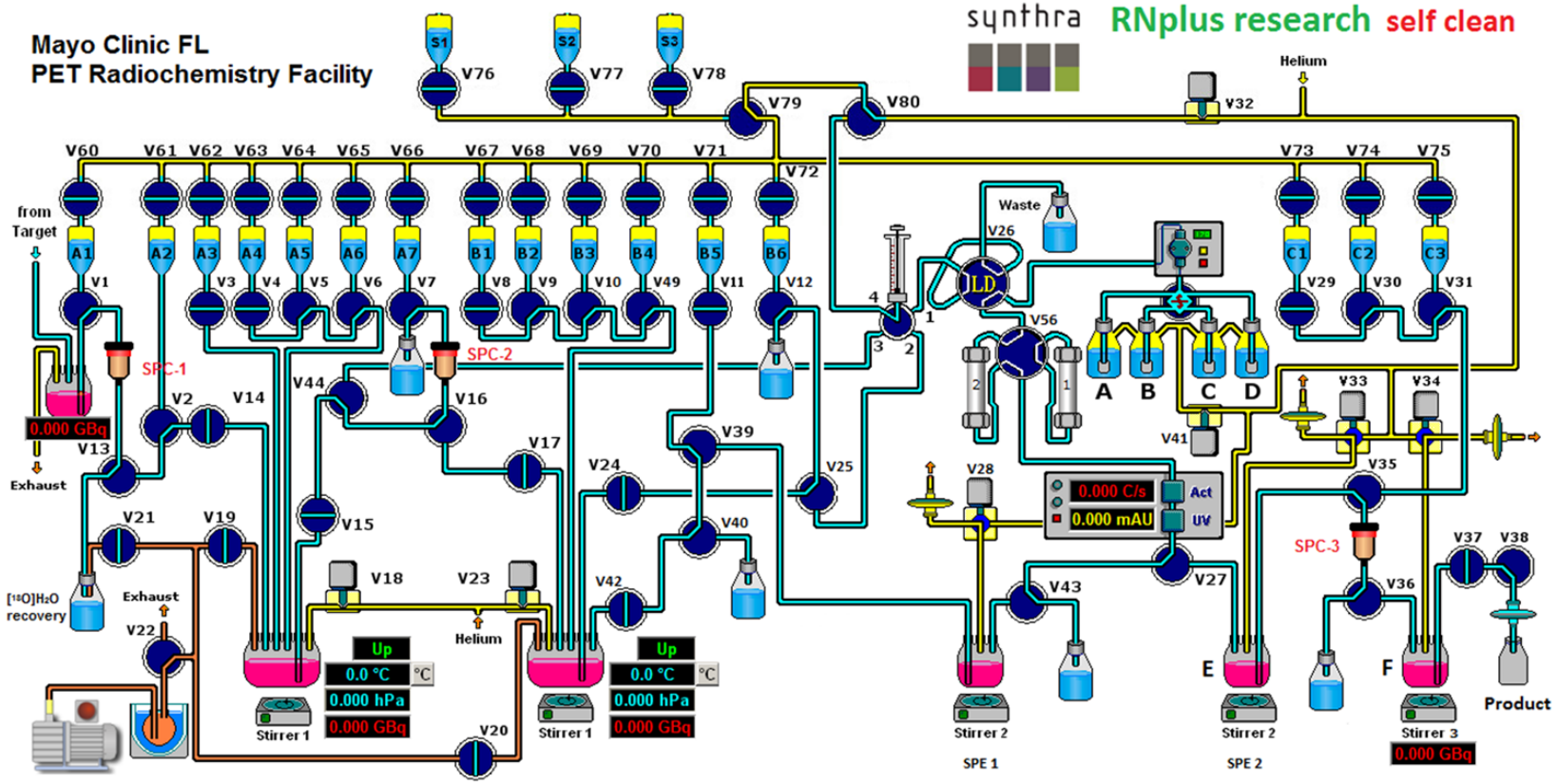


**Figure 2.** Diagram of RNplus Research module for [18F]Flortaucipir production. A1: 1 mL $K_{2.2.2}/K_2CO_3$ solution; A2: 1.5 mL deionized water; A3: 1 mg of AV1622 precursor dissolved in 1 mL DMSO; A4: 0.5 mL 3 N aqueous HCl; A5: 3.5 mL 0.5 N aqueous NaOH; A6: 5 mL deionized water; A7: 1.5 mL acetonitrile; C1: 10 mL sterile water for injection, USP; C2: 1.2 mL ethanol; C3: 2 mL 0.9% sodium chloride for injection, USP; E: 30 mL deionized water; F: 8 mL sodium chloride 0.9% USP; SPC-1: QMA cartridge; SPC-2: HLB cartridge; SPC-3: tC18 cartridge; HPLC eluent-A: 10 mM ammonium acetate; HPLC eluent-B: deionized water; HPLC eluent C: acetonitrile; S1: deionized water; S2: ethanol; and S3: acetone.

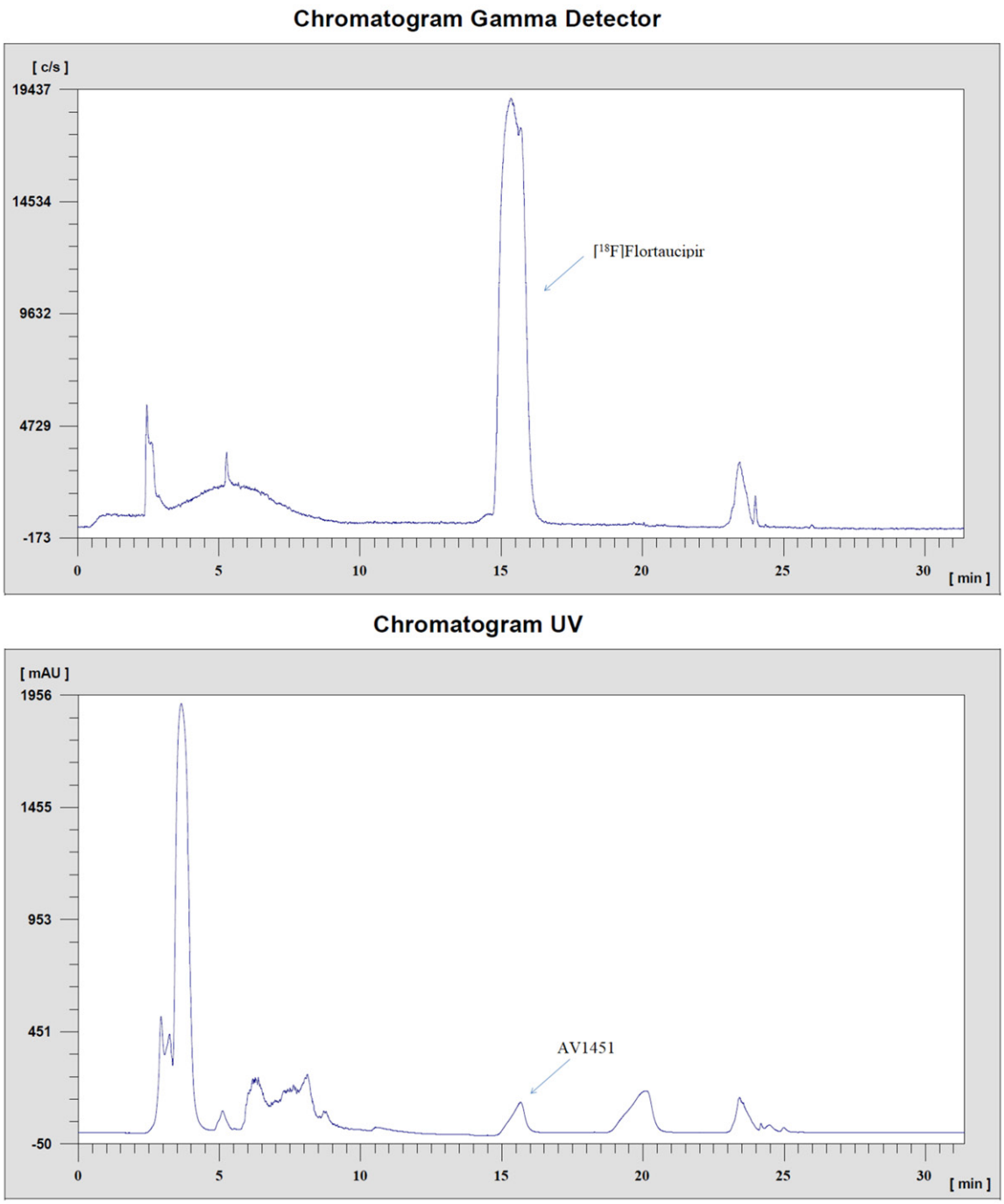


**Figure 3.** Representative semi-preparative HPLC chromatograms for purification of [18F]Flortaucipir.

vents content, residual $K_{2.2.2}$ concentration, molecular activity, radioactive concentration, filter integrity, endotoxin and sterility, conducted at the end of synthesis (EOS) and 8 h after EOS during the production validation runs, ensuring that the produced [18F]Flortaucipir meet the release criteria for human use. The detailed procedure for each QC test was described below.

*Appearance*

The final drug product in a multi-dose vial was observed visually behind leaded glass for any

color or particulate matter. Three validation batches of [18F]Flortaucipir were checked for color and clarity. Final product must be a clear, colorless solution, and free from particulate matter.

*Radionuclidic identity*

The half-life ($T_{1/2}$) was measured by placing a sample of drug product into a properly calibrated dose calibrator. The activities were recorded at multiple time points, at least 10 minutes apart from one another. Three validation batches of [18F]Flortaucipir were checked for radionuclidic identity by measuring the radioactivity over a 10-minute period. The $T_{1/2}$ was calculated by linear regression to assure that the $T_{1/2}$ = 105-115 minutes.

*Radiochemical identity*

A 20 μL sample of [18F]Flortaucipir product was injected into an HPLC system set up with a UV detector at 270 nm in line with a radioactivity detector as described above. The retention time ($R_t$) of the radioactive [18F]Flortaucipir was recorded and compared to that of the non-radioactive reference standard AV1451. Radiochemical identities were confirmed for three validation batches of [18F]Flortaucipir by HPLC analysis. Acceptable deviation in retention times of [18F]Flortaucipir and AV1451 standard was within ±10%.

*Radiochemical purity*

The same 20 μL injection of [18F]Flortaucipir product into the HPLC system for the determination of radiochemical identity was also used to measure radiochemical purity. All peaks appeared in the radioactivity chromatograph were integrated and the peak areas were recorded. Three validation batches of [18F]Flortaucipir were analyzed for radiochemical purity using radio-HPLC. Acceptable radiochemical purity from radio-HPLC analysis was ≥ 95%. To avoid the potential free [18F]fluoride ion in the final product which may not be detected by radio-HPLC system, Radio-TLC system with mobile phase of diethyl ether/methanol/ammonium hydroxide (V/V/V = 9:1:0.1) was introduced to detect radiochemical purity of [18F]Flortaucipir during validation process. Acceptable radiochemical purity from Radio-TLC analysis was ≥ 90%.

*Molar activity*

Three validation batches of [18F]Flortaucipir were analyzed for molar activity at EOS using the HPLC results. Recommended molar activity was ≥ 37 GBq/μmole (1000 mCi/μmole) at EOS.

*Chemical purity*

Chemical purity test includes residual solvent analysis by GC, $K_{2.2.2}$ residual test by thin layer chromatography (TLC), and non-radioactive mass of AV1451 and related impurities analysis using the UV mass tracer at 270 nm obtained as part of radiochemical identity and purity analysis by radio-HPLC. The amounts of residual solvents present in the product [18F]Flortaucipir were measured using GC. A 0.5 μL sample of product [18F]Flortaucipir was injected into the GC for assessing the presence of ethanol, acetonitrile, and DMSO. Acceptable solvent limits for ethanol, acetonitrile, and DMSO were ≤ 10.00% (w/v), ≤ 0.04% (w/v), and ≤ 0.50% (w/v), respectively. The amount of residual $K_{2.2.2}$ present in the drug product was measured by TLC by comparing with 50 μg/mL $K_{2.2.2}$ standard solution. [18F]Flortaucipir TLC spot must be less intense than the 50 μg/mL $K_{2.2.2}$ reference sample spot. Mass of AV1451 and related impurities in each batch of [18F]Flortaucipir were analyzed by HPLC method, the same as radiochemical purity analysis. The analytical HPLC method can quantitate the amount of reference standard AV1451 (retention time was about 4.4 min) and the precursor AV1622 (retention time was about 6.5 min), and other impurities present in the final product by comparing to a standard solution of AV1451 (2 μg/mL) and precursor AV1622 (4 μg/mL), respectively. In analytical HPLC results, there were a few unidentified UV peaks, which most of them were negligible and only one noticeable impurity peak among them was the deprotected precursor AV1622 at retention time of 3.1 min [18]. The acceptable mass of AV1451 was ≤ 20 μg/injected dose. The acceptable mass of total impurities including deprotected precursor AV1622 was 10% of allowable [18F]Flortaucipir cold mass, which must be ≤ 2.0 μg/injected dose using the same calculation as for AV1451 mass.

**Table 1.** Summary of batch analyses for [18F]Flortaucipir production

| [18F]Flortaucipir Batch | Run 1 | Run 2 | Run 3 |
|---|---|---|---|
| Starting [18F]Fluoride Activity (mCi) | 2741 | 3061 | 2891 |
| [18F]Flortaucipir Activity at EOS (mCi) | 426 | 507 | 427 |
| [18F]Flortaucipir Product Volume (mL) | 10.4 | 11.1 | 10.8 |
| Radiochemical Yield (%) (non-decay corrected) | 15.5 | 16.6 | 14.8 |

*Radionuclidic purity*

Radionuclidic purity testing of [18F]Flortaucipir was performed by gamma spectroscopy of a decayed sample using a germanium detector.

*pH*

The pH of the undiluted [18F]Flortaucipir product was measured using narrow-range pH test strips (0.3 units) and was visually confirmed by referencing the pH chart provided by the vendor.

*Filter membrane integrity (Bubble point test)*

The sterile filter for each batch of [18F]Flortaucipir production was checked using the bubble test at EOS to assure filter integrity. The integrity of the final drug product filter was evaluated as recommended by the filter manufacturer. The pressure was recorded from a calibrated pressure gauge.

*Bacterial endotoxin*

The bacterial endotoxin (BET) test was performed using the Endosafe Nexgen-MCS cartridge technology provided by Charles River Laboratories. Samples of the drug product were diluted with LAL water (1:100) and pipetted into a sterile, disposable LAL test cartridge with a sensitivity of 0.05 EU/mL that provided quantitative LAL results within 15-20 minutes. Final dose of the drug product must contains ≤ 175 EU/maximum injection volume.

*Sterility*

Sterility testing of [18F]Flortaucipir was completed retrospectively and initiated after the drug product had already been released for patient use. Briefly, sterility was initiated within 30 hours after EOS. A 0.2 mL sample of the undiluted drug product was inoculated in both tryptic soy broth (TSB) and fluid thioglycollate (FTM) media and incubated for 2 weeks alongside negative control samples. The TSB and FTM tubes were incubated in temperature-controlled incubators at 20-25°C and 30-35°C respectively. The sample was considered sterile if there was no visually detectable growth in both media 14 days later.

*Radioactive concentration (strength)*

The final drug product activity at EOS was measured using a properly calibrated dose calibrator. The final drug product vial was then weighed using a calibrated balance to determine an accurate product volume (calculated assuming the density of 1 g/mL). The activity was divided by the total volume of the drug product to calculate radioactive concentration. [18F]Flortaucipir strength was recorded on the batch record label. [18F]Flortaucipir strength was determined and recommended to be 0.3-1.9 GBq/mL (8.1-51 mCi/mL) at EOS.

## Results

*Radiosynthesis*

The radiosynthesis of [18F]Flortaucipir, which included [18F]fluoride receiving/activation, radiofluorination, deprotection, HPLC separation, SPE formation, final product delivery and sterile filtration, was fully automated processed by commercially available Synthra RNplus research module. Three consecutive batches of [18F]Flortaucipir production were successfully completed within defined specifications. The summary of three consecutive validation run results was presented in **Table 1**. Specifically, total 15.8-18.8 GBq (426-507 mCi) of [18F]Flortaucipir were obtained at EOS from starting activities of 101-113 GBq (2741-3061 mCi) with a total synthesis time of ~55 min. The non-decay corrected radiochemical yields were 14.8-16.6%. The final products were formulated in less than 10% ethanol in saline, with total volumes of 10.4-11.1 mL.

*Quality control*

The QC results showed that the produced [18F]Flortaucipir met all the release criteria for human use. As indicated in **Table 2**, all three batch products were clear, colorless solutions, and free from particulate matter. The pH and

**Table 2.** Summary of QC testing results for [18F]Flortaucipir validation runs

| Test | Acceptance Criteria | Validation Run Batch | | |
|---|---|---|---|---|
| | | Run 1 | Run 2 | Run 3 |
| Appearance | Clear, colorless solution, and free from particulate matter | Pass | Pass | Pass |
| Radionuclidic Identity | Half-life is 110 ± 5 minutes | 112.3 min | 109.5 min | 111.8 min |
| Radionuclidic purity | ≥ 99.5% of the observed peaks should correspond to the 0.511 MeV, 1.022 MeV, or compton scatter peaks of [18F]Fluoride | > 99.9% | > 99.9% | > 99.9% |
| Radiochemical Identity | $R_t$ deviation [18F]Flortaucipir ≤ ± 10% $R_t$ of AV1451 reference | 3% | 3% | 3% |
| Radiochemical Purity (HPLC) | Activity of [18F]Flortaucipir ≥ 95.0% total radioactivity | > 99.9% | > 99.9% | > 99.9% |
| GC Residual Solvents | EtOH ≤ 10.00% (w/v) | 7.71% | 7.53% | 6.51% |
| | MeCN ≤ 0.04% (w/v) | 0.00% | 0.00% | 0.00% |
| | DMSO ≤ 0.50% (w/v) | 0.00% | 0.02% | 0.00% |
| Chemical Purity* | AV1451 ≤ 20.0 μg/dose | 0.46 μg | 0.30 μg | 0.48 μg |
| | Total impurities ≤ 2.0 μg/dose | 0.16 μg | 0.14 μg | 0.18 μg |
| pH | 4.5-8.0 | 5.5 | 5.5 | 5.5 |
| $K_{2.2.2}$ | Size/intensity spot test ≤ 50 μg/ml | Pass | Pass | Pass |
| Molar Activity | ≥ 1.0 Ci/μmole @EOS | 6.8 Ci/μmol | 10.4 Ci/μmol | 6.7 Ci/μmol |
| Radioactive Concentration | 8.1 to 51.0 mCi/mL @EOS | 41.0 mCi/mL | 45.7 mCi/mL | 39.5 mCi/mL |
| Filter Integrity | ≥ 50 psi | Pass | Pass | Pass |
| Endotoxin | ≤ 175 EU/V** | Pass | Pass | Pass |
| Sterility | Pass | Pass | Pass | Pass |

*The presented mass of non-radioactive AV1451 and total impurities per dose were determined at 30 min post EOS. **V means the total volume of [18F]Flortaucipir product or maximum allowable 10 mL per dose.

half-life values were within the ranges of 4.5-8.0 and 105-115 min. From analytical HPLC results (**Figure 4**), the radiochemical purities were > 99.9%, and the radiochemical identities were within 3% deviation from the retention time of [18F]Flortaucipir and reference standard. The mass of AV1451 were 0.30-0.48 μg and the total impurities were 0.14-0.18 μg in the [18F]Flortaucipir doses targeted at 30 min post EOS. The molar activities were 247.9-384.8 GBq/μmol (6.7-10.4 Ci/μmol) at EOS. The radionuclidic purities were determined by no observed long-lived isotopes existence in the product after decay. The residual solvents in product were determined to be 6.51-7.71% ethanol and negligible amount of acetonitrile and DMSO on GC. The integrity of the final filter was demonstrated by a bubble-point filter test with holding ≥ 50 psi pressure. The formulated products were sterile and nonpyrogenic from the sterility and endotoxin results. Stability was evaluated by performing a full quality control at EOS, and followed by a repeated assessment of appearance, radiochemical identity/purity, chemical purity, pH, and bacterial endotoxin at the proposed expiration time of 8 hours after EOS. The [18F]Flortaucipir vial was stored in an inverted position at controlled room temperature for the duration of the expiration period. No leaks, sorption or degradation of the container closure were observed. A summary of the stability testing results at 8 h after EOS were given in Table S1. From the results, no significant changes were observed in pH, color, appearance, radiochemical/chemical purity, and endotoxin after 8 hours of room temperature storage. Based on these results, the expiration of [18F]Flortaucipir was set at 8 hours post EOS.

## Discussion

Tau protein has been shown to build up in the brains of patients with various neurodegenerative disease processes. PET imaging of tauopathies is an imaging technology that is transforming noninvasive assessment of neurodegenerative diseases, especially in AD. As a well-characterized radiotracer, [18F]Flortaucipir binds to the tau protein in the brain for noninvasive assessment of tauopathies in human

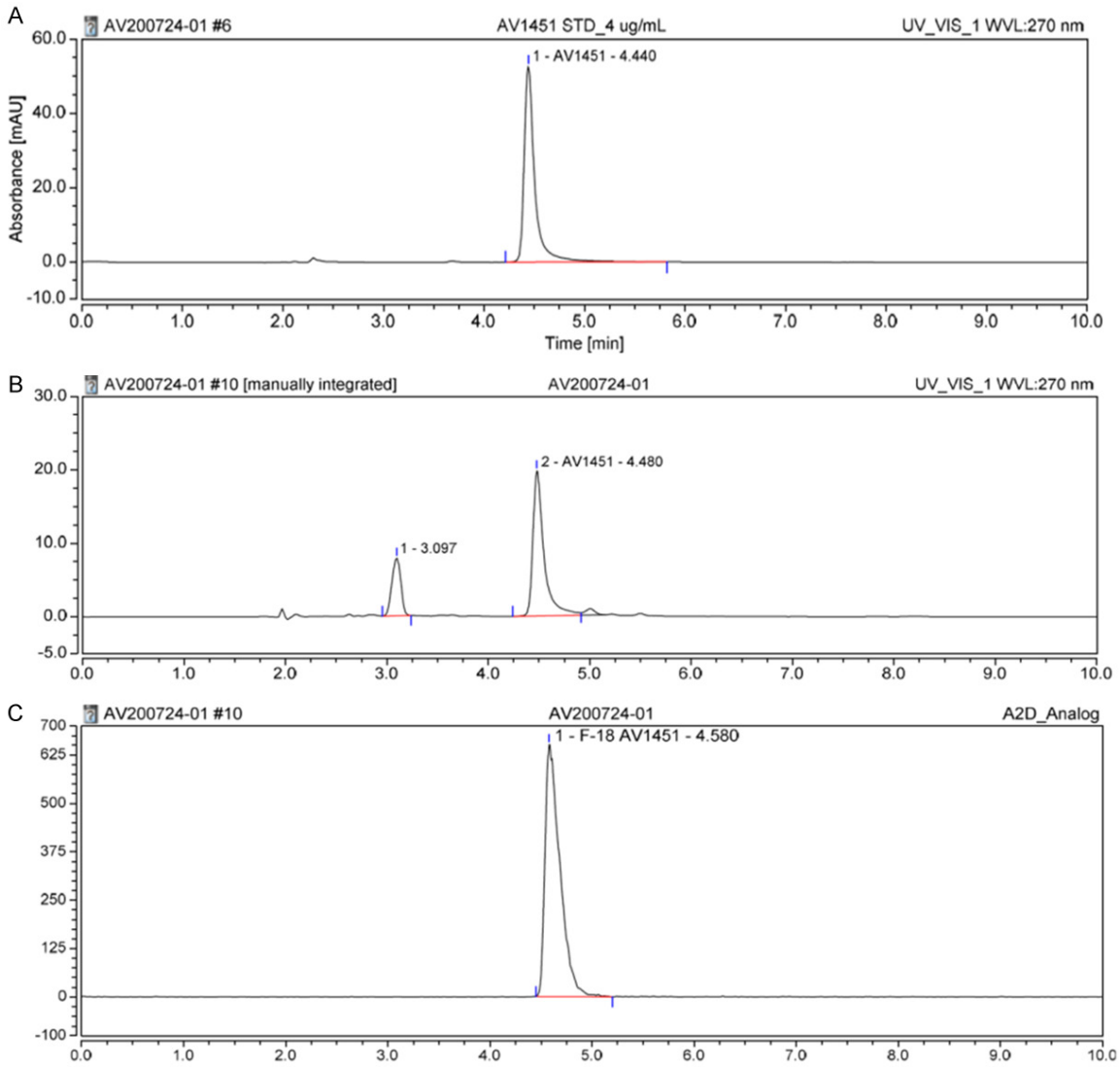


**Figure 4.** Representative analytical HPLC chromatograms of [18F]Flortaucipir and its reference standard. A. HPLC chromatograms for AV1451 reference standard (4 μg/mL); B and C. HPLC chromatograms for [18F]Flortaucipir.

with PET. The radiosynthesis of [18F]Flortaucipir in different module platforms were reported [13-20]. In this study, it was the first report for the radiosynthesis of [18F]Flortaucipir in Synthra RNplus Research module and we further optimized the production conditions by decreasing amount of precursor/reagents. The final product formulation contains approximate 11 mL solution of active ingredient [18F]Flortaucipir, and inactive ingredients including ethanol and sodium chloride in a single multi-dose sterile vial. In our three validation runs, 15.8-18.8 GBq (426-507 mCi) of [18F]Flortaucipir was obtained at EOS with uncorrected radiochemical yields of 14.8-16.6% in a total synthesis time of ~55 min, which were better than or comparable to these reported results from other commercial automated radiosynthesizers [13, 18].

*Radiosynthesis of [18F]Flortaucipir*

There were three precursors T-807P, N-Boc-T807P, and N-Boc-protected trimethylammonium precursor or named AV1622 available for radiosynthesis of [18F]Flortaucipir. Boc-protected precursors were more soluble and convenient for the radiosynthesis of [18F]Flortaucipir with better yields. The radiosynthesis of [18F]Flortaucipir with either one of Boc-protected

precursors were carried out via conventional two-step procedure of nucleophilic radiofluorination and acid-mediated deprotection or just with one-step nucleophilic radiofluorination, where the Boc group was also removed concomitantly in basic and thermal heating reaction conditions [13, 15, 16, 18, 19]. One-step radiofluorination with on-line deprotection method demonstrated its advantages for [18F] Flortaucipir preparation due to faster synthesis time and simple automation [13]. However, one study reported there was some difficulty in repeating the method [17]. Ensuring complete Boc-deprotection and better separation, we selected AV1622 for the two-step-one-pot radiosynthesis of [18F]Flortaucipir because this precursor and the method exhibited facile synthesis and convenient HPLC purification [20]. Two-step radiosynthesis of [18F]Flortaucipir with AV1622 were applied on microwave radiosynthesis module, Raytest® synthesis module, a Tracerlab FX N module with a > 10 mL reactor vessel [18-20]. To adopt this method to commercially available modules with smaller volume reactors, we modified the reaction and purification process by reducing the volumes by half, and applied to Synthra RNplus Research module successfully, which has a 10 mL reactor. Meanwhile, the precursor AV1622 mass was reduced from 1.8-2.0 mg to 1.0 mg to facilitate the HPLC purification and improve chemical purity without decreasing the radiochemical yield. In addition, an optimized low water content (3%) $K_{2.2.2}/K_2CO_3$ stock solution was formulated with 240 mg $K_{2.2.2}$ and 40 mg $K_2CO_3$ in 19.4 mL acetonitrile and 0.6 mL deionized water. Over 90% of [18F]Fluoride activity was eluted from QMA cartridge using 1 mL $K_{2.2.2}/K_2CO_3$ solution. The optimized dry-down steps only took 3 min at 70°C followed by 2 min at 100°C. Resulting dry [18F]fluoride was directly used for radiolabeling without extra addition of acetonitrile and azeotropic dryness. Desired radiolabeling yield and repeatability were achieved with this azeotropic dry process (n = 10). Using 1 ml of above $K_{2.2.2}/K_2CO_3$ solution for [18F]fluoride elution, it shortened azeotropic drying time to 5 min from typically 7-12 min, allowing fast radiosynthesis.

*QC of [18F]Flortaucipir*

The [18F]Flortaucipir product QC testing methods and acceptance/release criteria met both the FDA GMP guidance and the USP requirements for PET radiopharmaceuticals. During the QC method validation, there was a concern for potential [18F]fluoride leakage in the [18F]Flortaucipir product, where [18F]fluoride may retain on analytical C18 column and not be detected by HPLC [21]. We used Radio-TLC to verify the HPLC analysis of [18F]Flortaucipir radiochemical purity. In our hand, reliable radio-TLC results for [18F]Flortaucipir were obtained on aluminum oxide plates (Figure S1). The [18F]Flortaucipir sample was diluted with acetone and the TLC plate was developed with diethyl ether/methanol/28-30% ammonium hydroxide (v/v/v = 9:1:0.1) solution. As a result, less than 3% radioactivity was observed at the origin, and > 97% [18F]Flortaucipir activity was shown in the frontier of TLC plate [17], which confirmed the analytic HPLC method and the [18F]Flortaucipir radiochemical purity results from HPLC analysis were validated. Our QC results, similar to other reports, demonstrated the in-house automated prepared [18F]Flortaucipir product with high radiochemical purity and molar activity was a sterile, non-pyrogenic aqueous solution, suitable for intravenous administration.

## Conclusions

Automated production of [18F]Flortaucipir for human use was reproducibly achieved in Synthra RNplus Research module with desired radiochemical yield. [18F]Flortaucipir was obtained in high radiochemical purity and molar activity in a reasonable synthesis time. [18F] Flortaucipir validation run and quality control results demonstrated the efficacy of method for routine production under GMP compliance.

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## Disclosure of conflict of interest

None.

**Address correspondence to:** Hancheng Cai, Department of Radiology, Mayo Clinic, Jacksonville, FL, USA. E-mail: cai.hancheng@mayo.edu

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**Table S1.** Summary of Stability Testing Results of [$^{18}$F]Flortaucipir

| Test | Acceptance Criteria | Run 1 | | Run 2 | | Run 3 | |
|---|---|---|---|---|---|---|---|
| | | EOS | 8 hours | EOS | 8 hours | EOS | 8 hours |
| Appearance | Colorless and free from particulate matter | Pass | Pass | Pass | Pass | Pass | Pass |
| Radionuclidic Identity | Half-life is 110 ± 5 minutes | 112.3 min | 110.4 min | 109.5 min | 109.1 min | 111.8 min | 111.6 min |
| Radiochemical Identity | $R_t$ [$^{18}$F]Flortaucipir ± 10% $R_t$ of AV1451 std | 3% | 5% | 3% | 5% | 3% | 5% |
| Radiochemical Purity | Activity of [$^{18}$F]Flortaucipir ≥ 95.0% of total radioactivity | 100% | 100% | 100% | 100% | 100% | 100% |
| pH | 4.0-6.0 | 5.5 | 5.5 | 5.5 | 5.5 | 5.5 | 5.5 |
| GC Residual solvents | Ethanol ≤ 10.00% (w/v) | 7.71% | 6.95% | 7.53% | 6.90% | 6.51% | 6.33% |
| | MeCN ≤ 0.04% (w/v) | 0% | 0% | 0% | 0% | 0% | 0% |
| | DMSO ≤ 0.50% (w/v) | 0% | 0% | 0.02% | 0% | 0% | 0% |
| $K_{2.2.2}$ | $K_{2.2.2}$ Size/intensity spot test ≤ 50 µg/mL | Pass | Pass | Pass | Pass | Pass | Pass |
| Chemical Purity* | AV1-541 ≤ 20 µg/mL | 0.46 µg | 0.59 | 0.30 µg | 0.28 µg | 0.48 µg | 0.45 µg |
| | Total impurities ≤ 2 µg/mL | 0.16 µg | 0.21 | 0.14 µg | 0.15 µg | 0.18 µg | 0.17 µg |
| Endotoxin | < 175 EU/V** | Pass | Pass | Pass | Pass | Pass | Pass |

*The presented mass of non-radioactive AV1451 and total impurities per dose were determined at 30 min post EOS. **V means the total volume of [$^{18}$F]Flortaucipir product.

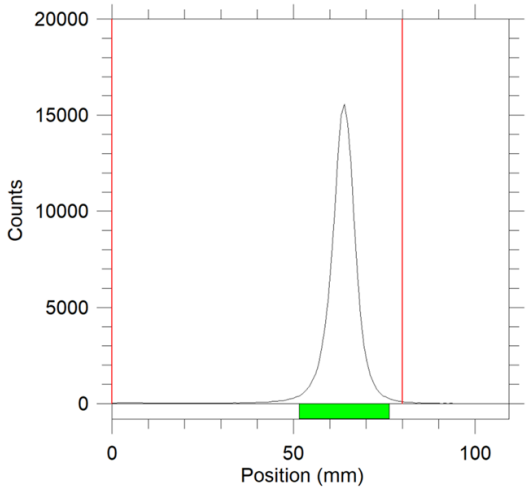


**Figure S1.** Representative radio-TLC chromatograms for [$^{18}$F] flortaucipir.