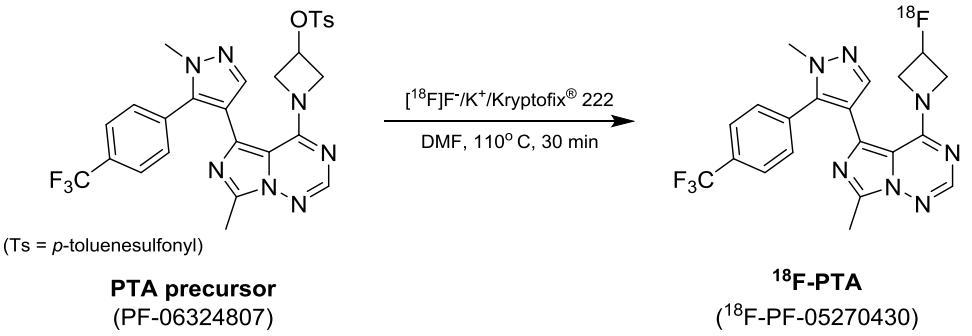
Supplemental File

Radiochemistry of ^{18}F-PF-05270430



Scheme 1. Radiosynthesis of ^{18}F-PF-05270430

Scheme 1 outlines the manufacturing procedure of the radioactive drug product ^{18}F-PF-05270430. The radiotracer was synthesized from the tosylate precursor PF-06324807, by a substitution reaction with ^{18}F-fluoride in the presence of Kryptofix® 222. ^{18}F-fluoride was produced via the ^{18}O(p,n)^{18}F nuclear reaction. This nuclear reaction is brought about by bombarding an enriched ^{18}O-water target assembly with a proton source using the GE PETtrace cyclotron. The target was routinely irradiated with a proton beam current of 35 μA for 30 min and provided about 1300-1500 mCi of ^{18}F-fluoride at the end of bombardment (EOB). The cyclotron produced ^{18}F-fluoride aqueous solution was transferred to the TRACERLab™ FX_{FN} automated synthesis module (GE Medical Systems), where the ^{18}F-fluoride ion was trapped on a Chromafix 30-PS-HCO_3 ^{18}F separation cartridge. Then ^{18}F-fluoride was eluted from the cartridge into the graphite reaction vessel of the FX_{FN} module with a solution of 13 mg of Kryptofix® 222 and 2 mg of K_2CO_3 in 1 mL of CH_3CN/water (9:1, v/v). The solvent was subsequently evaporated at 70º C for 5 min under reduced pressure (~33 kPa) and a stream of argon gas. A 1 mL aliquot of CH_3CN

was added to the reaction vessel and evaporation resumed at 70º C for 3 min, after which another 1 mL aliquot of CH_3CN was added and evaporation continued at 100ºC for another 4 min. The argon flow was then stopped and any remaining solvent was evaporated at 100ºC under reduced pressure (~8 kPa) for an additional 5 min leaving dry ^{18}F-fluoride. After air cooling to 60º C, 1.5 ± 0.1 mg of the precursor in 0.5 mL of anhydrous DMF was added to the reaction vessel, which was then sealed and heated at 110º C under stirring for 30 min.

Purification of the labeled product ^{18}F-PF-05270430 was accomplished in 2 stages: by solid-phase extraction, followed by preparative HPLC. First, the reaction mixture was cooled to 60ºC, diluted with 15 mL of 0.001 N HCl and loaded onto a Waters C-18 SepPak Light cartridge. The radiolabeled crude product was eluted of the SepPak cartridge with 1.5 mL solution of acetonitrile/USP grade absolute ethanol (2:1, v/v) into a receiving vial containing 3.5 mL of 0.1 M ammonium formate with 0.5% acetic acid, pH ~4.2-4.3. The combined solution mixture was purified by preparative HPLC. The preparative HPLC system used a semi-preparative column (Genesis C18, 4 μm, 10 × 250 mm) from Grace, Deerfield, IL. The column was eluted at a flow rate of 5 mL/min with a mixture of 33% acetonitrile and 67% 0.1 M ammonium formate solution containing 0.5% acetic acid pH of 4.2-4.3 (v/v). The eluent was monitored by a UV detector and a radioactivity detector. The desired product fraction was collected, diluted with 50 mL of de-ionized (DI) water, and passed through a second Waters C-18 SepPak Light cartridge. The SepPak cartridge was rinsed with 10 mL 0.001 N HCl. The radioactive product was recovered by eluting the SepPak with 1 mL of absolute ethanol (USP), followed by 3 mL of saline (USP), into a product vial containing 7 mL of saline (USP) and 40 μL of 4.2% sodium bicarbonate (USP). This mixture was then passed through a sterile membrane filter (0.22 μm) for terminal sterilization and collected in a sterile vial to afford a formulated IV solution ready for dispensing and injection.

A portion of the final formulated solution (~1 mL) was used for quality control tests. For determination of chemical purity, radiochemical purity, and specific radioactivity, an amount (5-100 μL) of the solution was withdrawn into an HPLC syringe and the amount of radioactivity (mCi) was assayed using a dose calibrator (Capintec). The solution was analyzed by HPLC (column: Gemini C18, 5 μm, 4.6 × 250 mm; mobile phase: 45% acetonitrile and 55% 0.1 M ammonium formate solution containing 0.5% acetic acid pH of 4.2-4.3 (v/v); flow rate: 1.5 mL/min; UV detector wavelength: 254 nm). The area of the UV peak associated with PF-05270430 was compared to a pre-defined standard mass curve to determine the mass (nmol). The specific activity (mCi/nmol) of ^{18}F-PF-05270430 is calculated as the ratio between the amount of radioactivity (mCi) and the amount of mass (nmol).

Plasma-free fraction

An ultrafiltration-based method was used for measuring the unbound portion (free fraction, f_P) of ^{18}F-PF-05270430 in plasma. A total of ~150 μCi of ^{18}F-PF-05270430 in a volume no greater than 0.1 mL was added to 6.0 mL of arterial blood sample taken immediate before injection. After 10 minutes incubation at room temperature, the spiked blood sample was centrifuged at 2930 g for 5 minutes to separate plasma. A 0.3 mL plasma sample was loaded onto the reservoir of the Millipore Centrifree® micropartition device in triplicate and centrifuged at 1228 g for 20 minutes. The f_P was determined by calculating the ratio of the mean radioactivity concentration of the ultrafiltrate (unbound) ^{18}F-PF-05270430 to the mean total activity in plasma.

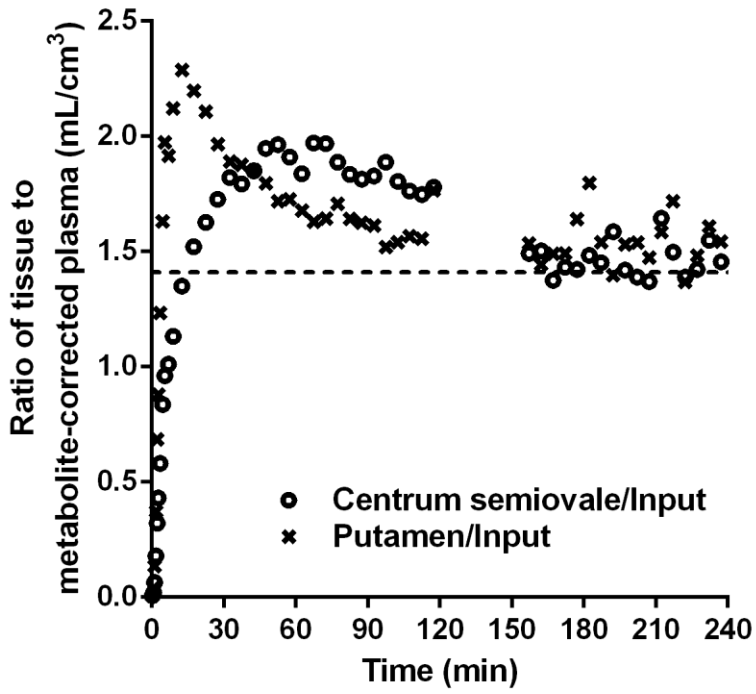
Determination of ligand metabolism in plasma

Plasma analysis of the radiotracer metabolism was performed from arterial blood samples collected at 5, 15, 30, 60, 90, and 120 minutes after injection (two more samples at 180 and 240 minutes for 240-min scans). Plasma metabolite analysis was performed using the column

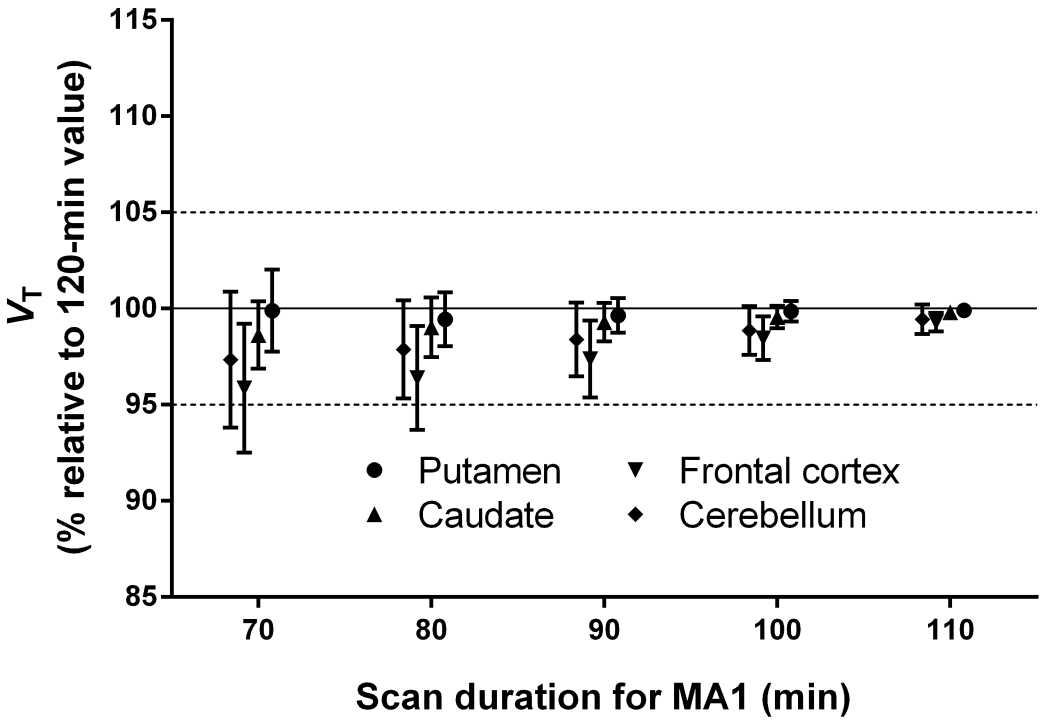
switching HPLC method to determine the parent fraction. In short, plasma samples treated with urea (8M) to eliminate plasma protein binding were loaded onto the capture column (19×4.6 mm) self-packed with Phenomenex SPE C18 Strata-X sorbent with 1% acetonitrile in water at 2 mL/min. The trapped activity was eluted through the analytical column [Phenomenex Luna(2) C18 analytical column (250×4.6 mm, 5 μm)] using 45% acetonitrile in 0.1M ammonium formate pH 6.4 at 1.55 mL/min. All the HPLC eluent was fraction-collected by an automated Spectrum Chromatography CF-1 fraction collection device. Activity in the blood, plasma, filtrated plasma-urea mix, filters, and fractions were counted with the well counter. The sample recovery rate, extraction efficiency, and HPLC fraction recovery were monitored. The unmetabolized parent fraction was determined as the ratio of the sum of radioactivity in fractions containing the parent compound to the total amount of radioactivity collected, and fitted with a sum of exponentials (bounder to not exceed 1.0) or an inverted gamma function. This fraction curve was also corrected for the time-varying extraction efficiency of radioactivity. The final plasma input function was calculated as the product of the total plasma curve and the parent fraction curve.

Uptake in the centrum semiovale

Based on V_T estimates, the distribution volume ratio between putamen (P) and centrum semiovale (CS) was 1.3 ± 0.2. Thus, at true equilibrium, we would expect the P:CS activity ratio to be 1.3. However, with a bolus injection, the regions are not at equilibrium. One approximation that can be helpful is to consider if the regions are at transient equilibrium, i.e., the ratio between regional actvity values is constant, and all regions clear at the same rate. In that case, there is an artificial contrast enhancement caused by plasma clearance which can have different magnitudes for different regions, depending on their kinetic parameters(*1*). For example, we can approximate this effect by adapting equation 3 from Carson et al.(*1*), for the 2TC model, and with $\beta = 0.012 \pm 0.003$ min^{-1} (the measured plasma clearance rate). In that case, the apparent volume of distribution (V_{app}) for putamen and centrum semiovale would both equal 1.4 mL/cm^3, so the P:CS ratio ($=V_{app}$(putamen)/V_{app}(centrum semiovale)) is expected to be 1.0 ± 0.1 Moreover, transient equilibrium is not yet reached by 90-120 min. Plotting the tissue to plasma ratio in both regions (see Supplemental figure 1 below), the putamen curve converged faster towards its transient equilibrium value of 1.4. Thus, at 90-120 min, the centrum semiovale had higher activity then the putamen due to its slower kinetics, which produce a larger transient equilibrium effect.



Supplemental Figure 1: The ratio of tissue curve to metabolite corrected plasma curve in centrum semiovale and putamen. The apparent (transient equilibrium) distribution volume was 1.4 for both regions and shown in horizontal dashed line.



Supplemental Figure 2: The effect of scan duration on estimation of V_T, using MA1 (t^*=40 min). Values were compared to the results from 120-min fits and plotted as mean and SD of the percent difference (n = 11).

Supplemental Table 1: Percentage difference of MA1 V_T estimates between 120-min and longer scan duration across three subjects (n = 6).

	[a]Percentage difference (mean ± SD) (%)				
Region	160 min	180 min	200 min	220 min	240 min
Putamen	0.2 ± 0.3	0.5 ± 0.7	0.6 ± 1.0	0.8 ± 1.1	1.0 ± 1.2
Nucleus accumbens	0.3 ± 0.7	0.9 ± 1.3	1.3 ± 1.5	1.5 ± 1.4	1.8 ± 1.4
Caudate	0.2 ± 0.2	0.9 ± 0.4	1.2 ± 0.4	1.5 ± 0.4	1.9 ± 0.6
Centrum semiovale	0.3 ± 0.4	1.2 ± 0.7	1.8 ± 0.8	2.2 ± 1.0	2.5 ± 1.2
Temporal cortex	0.4 ± 0.4	1.7 ± 0.6	2.5 ± 0.6	3.1 ± 0.8	3.7 ± 1.1
Occipital cortex	0.4 ± 0.4	2.0 ± 0.7	3.0 ± 0.9	3.9 ± 1.2	4.7 ± 1.7
Frontal cortex	0.7 ± 1.1	3.5 ± 2.6	4.8 ± 3.1	5.7 ± 3.4	6.8 ± 3.7
Parietal cortex	0.5 ± 0.8	3.2 ± 2.5	5.3 ± 3.8	7.2 ± 5.2	9.1 ± 7.0
Cerebellum	0.5 ± 0.6	2.6 ± 1.0	3.9 ± 1.0	5.0 ± 1.2	6.4 ± 1.0

[a] Percentage difference = (V_T(scan duration)/V_T(120-min) – 1) × 100

Supplemental Table 2: Test-retest reproducibility data in human

Radioligand	Target	Interval	BP_{ND} aTRV	References
^{11}C(*R*)-rolipram	PDE4	same day (n = 6), 45 ± 43 days (n = 6)	30	Zanotti-Fregonara et al, 2010(*2*)
^{11}C-LY2795050	KOR	same day	12	Naganawa et al, 2015(*3*)
^{18}F-MNI-659	PDE10A	N/A	9	Barret et al, 2014(*4*)
^{18}F-JNJ-42259152	PDE10A	26 ± 11 days	8	Van Laere et al, 2013(*5*)
^{18}F-FPEB	mGluR5	3-11 weeks	8	Park et al, 2015(*6*)
^{11}C-P943	5-HT1B	same day	6	Saricicek et al, 2015(*7*)

References

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