Radiosynthesis of *(S)*-^{18}F-THK5117

6-[(3-[^{18}F]fluoro-2-hydroxy)propoxy]-2-(4-methylaminophenyl)quinoline (*(S)*-^{18}F-THK5117) was produced according to good manufacturing practice at Uppsala University Hospital using fully automated synthesis equipment (Synthia, built in-house). The method was based on a previously described procedure with minor modifications (*11*). Cyclotron-produced [^{18}F]fluoride was concentrated using a quaternary methyl ammonium anion/carbonate solid phase extraction (SPE) cartridge (Waters #186004051) and then eluted with a solution of kryptofix-222 (10 mg) and K_2CO_3 (1.38 mg) in a water/acetonitrile mixture (100 µL/1 mL) into a glass vial (5 mL) equipped with a septum. The solvent was removed by heating the vial at 120°C under a stream of helium and reduced pressure for 800 s.

After the drying process, a solution containing the THP-protected tosylate *(S)*-THK5117-precursor (2-(4-methylaminophenyl)-6-[[2-(tetrahydro-2H-pyran-2-yloxy)-3-tosyloxy]propoxy]quinoline) (2 mg) in acetonitrile (700 µL) was added and the vial was heated at 110°C for 10 min. The THP protection group was then removed with aqueous HCl (400 µL, 1 M) for 2 min. Sodium acetate (210 µL, 0.19 M) was added to neutralize the pH and unreacted [^{18}F]fluoride was removed by passing the mixture through a neutral Al_2O_3-solid SPE cartridge (Waters, #023561). Semi-preparative high-performance liquid chromatography (HPLC) was used to purify and isolate the product. The column (ACE HL C18 5 µm 250 x 10 mm) was eluted with 10 mM ammonium acetate/acetonitrile (5/55) at 6 mL/min. The *(S)*-^{18}F-THK5117 retention time was approximately 6 min. The eluent was removed by rotary evaporation and the product was reformulated in ethanol (0.8 mL), hydroxypropyl-β-cyclodextrin (0.8 mL, 300 mg/mL) and phosphate buffer at pH 7.4 (7.2 mL, 0.1 M), and filtered through a 0.22 µm sterile filter.

The specific activity, radiochemical purity and chemical purity of the product were assessed by analytical HPLC equipped with radio and ultraviolet (310 nm) detectors and the identity was determined by comparison of retention time with authentic *(S)*-THK5117 reference compound. The column (Gemini NX C18, 5 µm, 250 x 4.6 mm, Torrance, CA, USA) was eluted with A:10 mM ammonium acetate and B:acetonitrile (40% B for 6.5 min; 40%-100% B in 1.5 min; 100% B for 3.5 min) at 1 mL/min. The *(S)*-^{18}F-THK5117 retention time was approximately 5.6 min. Enantiomeric purity was assessed by analytical HPLC using a chiral column (Kromasil AmyCoat 250 x 4.6 mm) eluted with a mixture of heptane/ethanol/diethylamine (75/25/0.1) at 3 mL/min. The *(S)*- and (*R)*-enantiomer retention times were 5.3 min and 6.3 min, respectively.

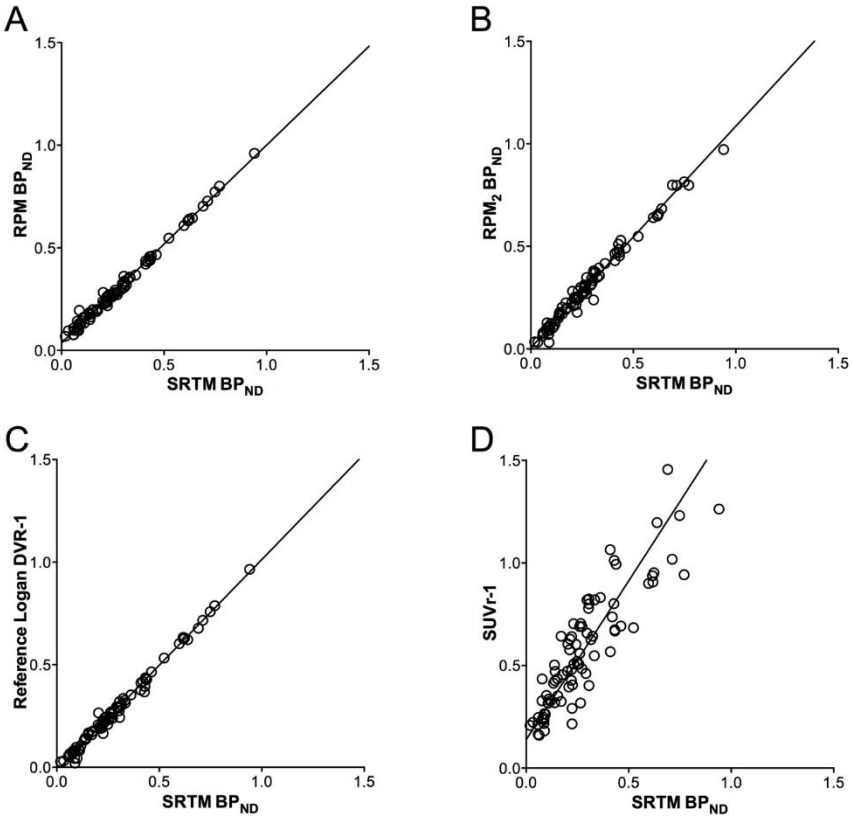
Metabolite analysis

Sample Preparation

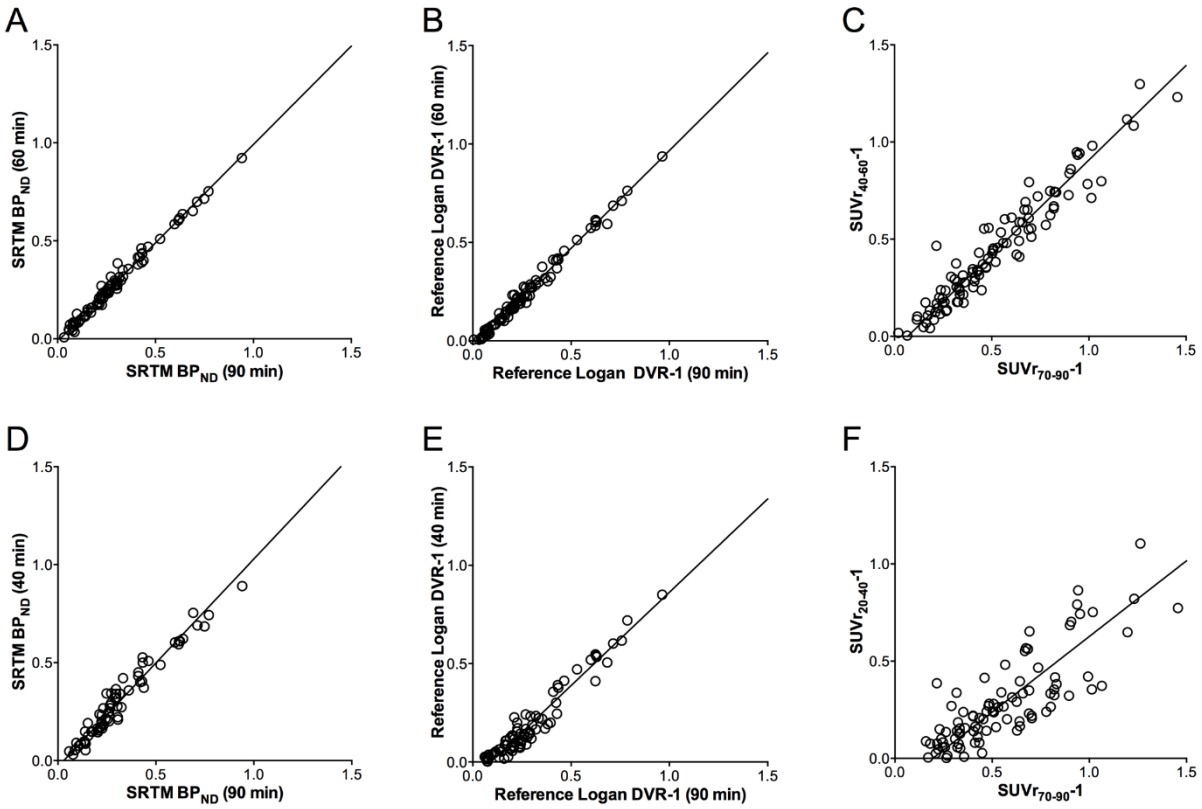
Each blood sample was centrifuged at 3000 x g for 2 minutes at 4°C (Beckman Allegra X-22R Centrifuge, Palo Alto, USA). Samples were taken from plasma (2 x 0.6 mL) and equal volumes of acetonitrile were added to precipitate proteins. The mixtures were centrifuged at 16000 x g at 4 °C for 1 min (Eppendorf 5415R centrifuge, Eppendorf AG, Hamburg, Germany). The supernatant was filtered through a 0.2 µm nylon membrane (Corning Incorporated, Corning, NY, USA) by centrifugation at 16000 x g at 4°C for 1 min and authentic *(S)*-THK5117 reference compound (10 µl, 0.1 mg/mL) was added to the mixture to aid identification of the parent fraction during HPLC separation. The sample preparation recovery was determined by measuring the radioactivity in the plasma, filters and pellet.

Analysis

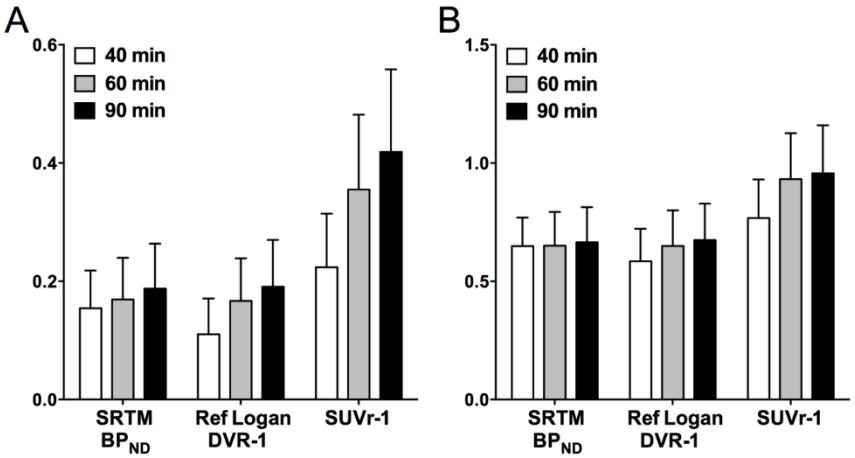
The metabolites were fractionated using a binary pump HPLC system (Gilson, Middleton, USA) equipped with a robotic liquid handler (ASPEC Gilson), an ultraviolet detector (310 nm) and a radio detector (Radiomatic 610TR, Packard, USA). The sample (1.8 mL) was injected onto a semi-preparative HPLC column (Gemini C18, 110 Å, 5 µm, 250x10 mm, Phenomenex) equipped with a guard column (C18 SecurityGuard, 10x10 mm, Phenomenex). The column was eluted at a flow rate of 6 mL/min with acetonitrile-50 mM ammonium acetate pH 5.8 (55:45, v/v). The outlet from the detector was connected to a switching valve on the arm of the liquid handler to enable automatic fraction collection. Three fractions were collected and the radioactivity in each fraction was measured by a well-type scintillation counter.



Supplemental Figure 1 *VOI-based SRTM BP_{ND} values versus A) RPM BP_{ND} B) RPM_2 BP_{ND}, C) reference Logan DVR-1 and D) SUVr-1 values based on the parametric images. The lines are orthogonal regressions.*



Supplemental Figure 2 *Comparisons between 90 and 60 min scan duration for A) SRTM BP_{ND}, B) reference Logan and C) SUVr-1. D-F) Comparisons between 90 and 40 min scan duration for D) SRTM BP_{ND}, E) reference Logan DVR-1 and F) SUVr-1. The lines are orthogonal regressions.*



Supplemental Figure 3 *Comparisons between outcome parameters based on 90, 60 and 40 min scan duration for temporal cortex (A) and putamen (B).*