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GMP Production of [18F]FE-PE2I on a TRACERLab FX2 N synthesis module, a Radiotracer for in vivo PET Imaging of the Dopamine Transport

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Abstract

Background

Parkinson's disease is a neurodegenerative disorder that is characterized by a degeneration of the dopaminergic system. Dopamine transporter (DAT) positron emission tomography (PET) imaging has emerged as a powerful and non-invasive method to quantify dopaminergic function in the living brain. The PET radioligand, [^{18}F]FE-PE2I, a cocaine chemical derivative, has shown promising properties for in vivo PET imaging of DAT, including high affinity and selectivity for DAT, excellent brain permeability, and favorable metabolism. The aim of the current study was to scale up the production of [^{18}F]FE-PE2I to fulfil the increasing clinical demand for this tracer.

Results

Thus, a fully automated and GMP-compliant production procedure has been developed using a commercially available radiosynthesis module GE TRACERLab FX2 N. [^{18}F]FE-PE2I was produced with a radiochemical yield of 39 ± 8% (n = 4, relative [^{18}F]F^- delivered to the module). The synthesis time was 70 minutes, and the molar activity was 925.3 ± 763 GBq/μmol (250 ± 20 Ci/μmol). The produced [^{18}F]FE-PE2I was stable over 6 hours at room temperature.

Conclusion

The protocol reliably provides a sterile and pyrogen–free GMP-compliant product.

Background

The dopamine transporter (DAT) is a plasma membrane protein expressed exclusively on presynaptic dopaminergic neurons in the central nervous system (CNS). It is responsible for regulating the synaptic concentration of dopamine out of the synaptic cleft into the neurons. DAT imaging in the nigrostriatal system is a well-established tool for the evaluation of dopaminergic function in neurodegenerative disorders, e.g., Parkinson's disease (PD) and Parkinson´s plus-syndromes or the atypical parkinsonians (APS). [1–2]

Until now, DAT imaging has predominantly been performed on a daily clinical practice using the commercially available SPECT (single-photon emission computed tomography) radiopharmaceutical, [^{123}I]FP-CIT ([^{123}I]-ioflupane, DaTSCAN, GE HealthCare), an approved tracer for PD diagnostics.[3] Moreover, FP-CIT SPECT is also being used to differentiate neurodegenerative disorders from essential tremor, drug-induced or vascular forms of parkinsonism but cannot differentiate between PD and APS.

Positron emission tomography (PET) imaging, on the other hand, is a more sensitive technique to measure the density and activity of DAT in the brain, which could potentially be useful for diagnosis and evaluation of possible treatments.[4] Several radioligands for imaging DAT have been reported and applied for PET in human subjects, most of which have been derivatives of cocaine, [^{11}C]PE2I [5], [^{11}C]β-CIT [6], [^{11}C]β-CIT-FE [7], [^{18}F]β-CFT [8], [^{18}F]FECNT [9], and [^{18}F]LBT-999 [10]. The ^{18}F-labelled analogue of PE2I, (E)-N-(3-iodoprop-2-enyl)-2β-carbofluoroethoxy-3β-(4'-methyl-phenyl) nortropane ([^{18}F]FE-PE2I), has shown excellent properties for *in vivo* imaging of DAT, which includes, high affinity and selectivity, excellent brain permeability, favorable metabolism, and shows appropriate in vivo kinetics.[11–13] The high affinity of [^{18}F]FE-PE2I even allows the visualization and quantification of ligand binding to DAT in the substantia nigra.

The synthesis of [^{18}F]FE-PE2I was first described by Schou et. al., via a two-step and two-pot procedure. Although, the yield and purity of [^{18}F]FE-PE2I (RCY of 7% and a RCP > 95%) was sufficient for the initial Non-Human Primate (NHP) PET evaluation, the synthesis procedure was deemed unsuitable for routine clinical productions. [11–12] Therefore, a simplified, one-step radiofluorination procedure was later presented by the same research group (Scheme 1). This semi-automated method provided [^{18}F]FE-PE2I in good and reproducible yields (RCY = 20%).[14]

Moreover, a recent study adapted this one-step synthesis method of [^{18}F]FE-PE2I for an automated cassette-based radiochemistry module (Synthera®$^{+}$, IBA).[15]

The goal of the current work was to enable large-scale production of [^{18}F]FE-PE2I to meet the increasing clinical demand for an ^{18}F-labelled DAT PET imaging agent. Herein, we report the fully automated radiosynthesis of [^{18}F]FE-PE2I performed under good manufacturing procedure (GMP) conditions using a commercial radiofluorination module (GE TRACERLab FX2 N) and its comprehensive validation for clinical routine human use.

Methods

Experimental and materials

All chemicals and reagents were obtained from Sigma-Aldrich and were used as received without further purification. The precursor tosylethyl-PE2I was purchased from Pharmasynth AS (Estonia). Solid-phase extraction cartridges: Sep-Pak Accell Plus QMA Plus Light Cartridge and tC18 Plus short Cartridge were purchased from Waters Corporation (Milford, Massachusetts, USA).

High-performance liquid chromatography (HPLC) analysis of compounds was performed on a Poroshell 120 EC C-18, 3 × 150 mm, i.d. 2.7 µm column on an Agilent 1260 HPLC system (UV absorbance 220 nm) using TFA 0,1%/ACN as eluent (gradient elution) with a flow of 0.5 mL/min. For more information, please refer to the section "Quality control procedure".

The purification of [^{18}F]FE-PE2I is performed with a semi preparative ACE HPLC column (5µm C-18 HL, 10x250nm, Advanced Chromatography Technologies) using ACN:H_2O:TFA 175:325:0,5 (v/v/v) mobile phase (isocratic elution). The HPLC purification system consists of a pump (Sykom), an automated sample injection equipped with a 2 mL stainless-steel loop. UV detector from Knauer and a gamma radioactivity PIN diode detector.

Radio-thin layer chromatography (radio-TLC) analyses were run on TLC Silica gel 60 F_{254}, glass plates, 2.5x7.5 cm (Merck) (stationary phase) using acetonitrile and 0.1M citrate buffer, pH 5.0 (1:1, v/v) as a mobile phase. Radioactivity spots were detected using an automatic radio-TLC scanner (Scan-RAM™ PET/SPECT radio-TLC scanner).

The Gas chromatography (GC) method is developed for a 30 m long Res-Solv capillary column having 0.53 mm inner diameter and a 1.0 µm film. The flame ionization detector (FID) is used as detector to analyze ions formed during combustion of organic compound in synthetic air and hydrogen gas. The injection volume was 2µL.

The split ratio was 1:80 and the inlet and detector temperature were 250°C. The temperature program: 35°C for 3.5 minutes after injection, ramp to 240°C at 70°C/minute, hold at 240°C for 3 minutes, cool to 35°C. The GC is performed to verify a separation between solvents to be analyzed, i.e. DMSO, acetonitrile and ethanol.

The synthesis method sequence for GE TRACERLab FX2 N system was developed in-house at Karolinska Radiopharmacy department, Karolinska University Hospital. Production of [^{18}F]FE-PE2I was performed in a class C cleanroom laboratory and the GE TRACERLab FX2 N synthesizer is located in a BBS hotcell (Comecer). Two product vials are assembled in a laminar airflow workbench with a sterile product filter and a ventilation filter to receive the radiolabeled tracer.

The product is finally released by an onsite QA/QP prior to use in human PET studies.

[^{18}F]FE-PE2I was approved by the Swedish Medical Product Agency for clinical examinations in patients on a yearly license. PET/CT imaging was performed on a GE Discovery MI PET/CT (GE Healthcare, Milwaukee, WI).

Patients received a dose of 200 MBq [^{18}F]FE-PE2I bolus intravenous injection and then allowed to rest for 30 minutes before being placed supine and head-first, in the PET/CT scanner. A low-dose CT for attenuation correction was performed (100 kV, 0 mAs, slice thickness of 3.75 and FOV: 700 mm), before a static PET acquisition acquired in list-mode for 12 minutes. PET data were reconstructed by the ordered-subsets expectation maximization (OSEM) algorithm (3 iterations, 34 subsets) and a 3 mm Gauss filter, after application of all suitable corrections such as those for photon attenuation, scattered radiation, time-of-flight (TOF), point spread function (PSF) and radioactive decay of the [^{18}F]FE-PE2I.

For the visual assessment and image interpretation, the reconstructed PET-data were analyzed using the commercially available Hermes software (Hermes Medical Solution, Sweden) https://www.hermesmedical.com/our-software/

Results

Synthesis of [^{18}F]FE-PE2I

A schematic diagram of the GE TRACERLab FX2 N radiosynthesis module used for the synthesis of [^{18}F]FE-PE2I is shown in Fig. 1. The in-house developed reaction sequence for [^{18}F]FE-PE2I (Scheme 1) involves three main steps: (i) the initial azeotropic drying of [^{18}F]F^{-}; followed by (ii) nucleophilic fluorination of the precursor compound; and finally (iii) HPLC purification and formulation of the final product. The synthesis module was operated in the following sequences with numerical references to vials (**1**–**20**) in Fig. 1:

1. At the end of bombardment, aqueous [^{18}F]fluoride ([^{18}F]F^{-}, ~ 50–83 GBq) was produced following the nuclear reaction: $^{18}O(p, n)^{18}F$ using a General Electric Medical PETtrace 800 cyclotron (16.4 MeV). The short-lived radionuclide was transferred from the target to a collection vial **15** via a stream of helium gas (6.0, AGA).
2. The aqueous [^{18}F]F^{-} solution was transferred from the collection vial **15** via V10–11 over a pre-activated (10 mL 0.5 M potassium carbonate and 10 mL > 16 MΩ water) Sep-Pak Accell Plus QMA Plus Light Cartridge (Waters). [^{18}F]F^{-} was quantitively trapped on the QMA cartridge and [^{18}O]H_2O was recovered in a recovery vial **16**.
3. The trapped [^{18}F]F^{-} (~ 50–60 GBq) are eluted from the QMA using 1.0 mL of a Kryptofix eluting solution (4.7 mg Kryptofix®222, 0.9 mg K_2CO_3, 40 µL > 16 MΩ water, 960 µL acetonitrile), preloaded into vial **1** and delivered to the reaction vial **17**.
4. The [^{18}F]F^{-} mixture in vial **17** was first dried azeotropically at 85°C under N_2 flow and vacuum for 7 min, and later at 110°C under N_2 flow and vacuum for another 5 min. The reaction vial was then cooled to 60°C prior to the next step.
5. The precursor solution (1.0 mg tosylethyl-PE2I dissolved in 1.5 mL DMSO) is preloaded into vial **3** and further added to the reaction vial **17**. The reactor is sealed and heated to 140°C for 150 seconds. After completed reaction, the reactor was cooled to 60°C prior to the next step.
6. The crude reaction mixture was then diluted with 1.5 mL of mobile phase (35:65, acetonitrile:0.1% trifluoracetic acid with 0.5 mg/mL sodium ascorbate) and 2.0 mL sodium ascorbate solution (5 mg/mL in sterile water), which was added from vial **5** to the reaction vessel **17**, prior to HPLC purification.
7. The content of vial **17** was first transferred into an intermediate vial **18**, before it was delivered to the HPLC loop (5 mL) via a fluid detector. The solution was further injected into a semi-preparative HPLC column (ACE 5, C18-HL, 250 x 10 mm i.d., 5 µm), and eluted with mobile phase (35:65 acetonitrile :

0.1% trifluoracetic acid with 0.5 mg/mL sodium ascorbate) at a flow rate of 5 mL/min. The elute was monitored by UV (λ = 254 nm), and a radioactivity detector connected in series.

8. A typical semi-preparative HPLC chromatogram is displayed in Fig. 2 using an isocratic elution of 35:65 acetonitrile: 0.1% trifluoracetic acid with 0.5 mg/mL sodium ascorbate at a flow rate of 5 mL/min, and a semi-preparative column ACE 5 C18-HL, 250 x 10 mm i.d., 5 µm. The fraction containing the desired product, [^{18}F]FE-PE2I (retention time ≈ 25 min), was collected into a collection vessel **19**, which was preloaded with 40 mL sodium ascorbate solution (5 mg/mL in sterile water).
9. The resulting solution is then transferred via V17 and V15 over a pre-activated (10 mL ethanol 99.5% and 10 mL of sodium ascorbate solution (5 mg/mL in sterile water)) Sep-Pak tC18 Plus short Cartridge (Waters). [^{18}F]FE-PE2I was trapped on the tC18 cartridge and immediately thereafter washed with 10 mL sodium ascorbate solution (5 mg/mL in sterile water), preloaded into vial **14**.
10. [^{18}F]FE-PE2I was eluted using 1.5 mL ethanol, preloaded into vial **13** and delivered to final mixing vial (**20**) which had been preloaded with 233 mg sodium ascorbate dissolved in 10 mL of saline (0.9% NaCl, pH 4.5–7.0). The final solution further diluted with 117 mg sodium ascorbate in saline from vial **12**.
11. Finally, the formulated product (volume = 16.5 mL, ~ 9% ethanol in saline) was delivered into two separate product vials via two different sterile filters (0.22 µm sterile Millex-GV filter, Millipore). The final volume obtained for product vial 1 and product vial 2 was 8 mL and 6 mL, respectively. This was accomplished by applying a constant helium pressure (1 Bar) to the final mixing vial **20** for a specific timeframe (product vial 1 = 50 s; product vial 2 = 40 s). In our setup, with 1 Bar input pressure and a tube length of approximately 0.5 m, a flow rate of ~ 10 mL/min was generated.

Quality control procedure

Table 1
Specifications for [18F]FE-PE2I Solution for Injection

Parameters	Product specifications	Equipment
Appearance	Clear or slightly yellow. Free of particles	Visual inspection
Filter integrity	≥ 3.5 bar	Bubble point tester
pH	4.5–8.0	pH-meter or pH indicator
Product identity [^{18}F]FE-PE2I	$\lvert Rt_{RD} - Rt_{UV} \rvert \leq 60$ s	HPLC
Chemical purity FE-PE2I	Mass limit ≤ 5 µg/patient dose*	
Chemical impurities	Mass limit ≤ 5 µg /patient dose*	
Radiochemical impurity Impurity = B [^{18}F]fluoride	≤ 5%	TLC
Total radiochemical purity [^{18}F]FE-PE2I RCP_{Tot} = (100 – B) x T	≥ 93%	HPLC and TLC
Mass limit	≤ 5 µg FE-PE2I and ≤ 5 µg impurities per 200 MBq (patient dose)	HPLC
Residual Kryptofix 222 content	< 0.14 mg/mL	Spot test
Bacterial endotoxins	< 11.5 IU/mL	Endosafe
Acetonitrile	< 0.27 mg/mL	GC
DMSO	< 3.3 mg/mL	
Ethanol	< 80 mg/mL	

Parameters	Product specifications	Equipment
Sterility	Sterile, 0 CFU	Direct inoculation by an approved contractor
Radionuclidic identity	Principle peak at 511 keV, possible summation peak 1022 keV. Not more than 0.1% radioactivity from total, determined 24 h after EOS	HPGe detector
Radionuclidic identity Half-life [^{18}F]	105–115 min	Dose calibrator
Radiochemical stability	$RCP_{Tot} \geq 93\%$ up to 6 h EOS	HPLC
Shelf-life	Batch specific, calculated time where a patient dose exceeds mass limit	HPLC
* A patient dose is defined as 200 ± 20 MBq (for a 70 kg patient) with a mass limit of ≤ 5 µg/patient dose of FE-PE2I and ≤ 5 µg /patient dose of impurities, calibrated at the time of injection.		

The quality control of [^{18}F]FE-PE2I Solution of Injection was performed using validated analytical methods. The specifications, tests and frequency used is summarized in Table 1.

The product specifications are based on a patient dose of 200 ± 20 MBq (for a 70 kg patient) with a mass limit of ≤ 5 µg/patient dose of FE-PE2I and ≤ 5 µg /patient dose of impurities, calibrated at the time of injection. Specifications for kryptofix and endotoxins are based on a maximum injection volume of 15 mL.

The non-radioactive impurities in the product formulation can potentially compete in binding with the biological target with our desired radiotracer. In addition, due to some toxicity concerns measuring the exact amount mass of injected product (the mass is important for further molar activity calculation) into the subjects is an important factor. Therefore, developing a sensitive and reliable HPLC method for QC is crucial. The HPLC analysis was performed utilizing an Agilent 1260, mobile phase A: TFA 0.1% and B: Acetonitrile, using the following gradient; 0–1 min A:60 and B:40, 1 to 8 min A:20 and B:80, 8 to 10 min A:80 and B:20, 10–15 min A:80 and B:20, flow = 0.5 mL/min, column; Poroshell 120 EC C-18, 3 × 150 mm, i.d. 2.7 µm column, injection volume = 50 µL, λ = 220 nm. The developed method presented a proper linearity in the range of 0.5 to 10 µg/mL. The obtained limit of quantification (LOQ) and limit of detection (LOD) for this method were 0.98 µg/mL and 0.3 µg/mL, respectively. The represented chromatograms for the system suitability test (SST) and [^{18}F]FE-PE2I analysis are shown in Fig. 3. The SST was combination of; FE-PE2I, Desmethyl-PE2I (potential by-product) and Tosylethyl-PE2I (precursor). The coefficients of determination (R^2) in the aqueous sample were 0.9996. The accuracy values of the QC samples at three

different concentration levels (high, middle, and low) varied in the range of 1.02–3.74% (n = 9). The inter-day and intra-day precision were lower than 3.66 and 2.84%, respectively.

Furthermore, a radio-TLC analysis has been performed to determine the percentage of radiochemical impurity, [^{18}F]fluoride in the product (Fig. 4). Based on that result, the total RCP of the [^{18}F]FE-PE2I Solution of Injection is calculated following the formula below:

$$RCP_{Tot} = (100 - B) \times T$$

B: Free radioactive [^{18}F]fluoride (%) analyzed using TLC analysis.

T: Proportion of the radioactivity due to [^{18}F]FE-PE2I using the HPLC analysis.

The gas chromatography is performed to verify and quantify the amount of residual solvents i.e DMSO, acetonitrile and ethanol in [^{18}F]FE-PE2I product (Fig. 5).

Discussion

Radiosynthesis of [^{18}F]FE-PE2I was automated using a commercial radiofluorination module (GE TRACERLab FX2 N), specifically designed for fluoride-18 radiolabeling with an HPLC purification system. We adapted the previously semi-automated protocol reported by Stepanov et. al.[14] to accommodate the commercial radiosynthesis unit, as well as established quality control procedures that would satisfy the EAM regulatory requirements for GMP production and human PET imaging studies. Radiolabeling was performed in a single-step by nucleophilic substitution reaction of the tosylethyl-PE2I precursor compound using azeotropically dried potassium cryptand [^{18}F]fluoride complex ([^{18}F]KF/$K_{2.2.2}$) dissolved in dimethyl sulfoxide. The resulting mixture was heated at 140°C for 150 seconds. After the completed reaction, the crude product solution was further purified by a semi-preparative HPLC. HPLC purification of [^{18}F]FE-PE2I was performed on a semi-preparative ACE 5 C18-HL column, using a mixture of water, acetonitrile, trifluoracetic acid, and sodium ascorbate (0.5 mg/mL) as the eluent. The desired fraction was collected and diluted with 5 mg/mL sodium ascorbate in sterile water and finally reformulated using SPE to produce [^{18}F]FE-PE2I in a reproducible decay-corrected radiochemical yield of 39 ± 8% (n = 4, relative [^{18}F]F^- delivered to the module). It is important to note that during the synthesis procedure development, it was found that the addition of sodium ascorbate to the purification and reformulation steps as well as to the formulated product was necessary to obtain a product with high stability and purity. Removing sodium ascorbate in any part of the procedure resulted in rapid decomposition, likely attributed to radiolysis. Nonetheless, using the above-described conditions, [^{18}F]FEPE2I was obtained in high radiochemical purity (> 95%) and a molar activity (A_m) of 925.3 GBq/μmol (250 Ci/μmol) at the end of synthesis. The overall synthesis time was 70 minutes including formulation. Although radioactive losses could likely be minimized by further optimization of the fluorination, (10–15 GBq, 270–405 mCi) were prepared in a form suitable for human use (Table 2).

Table 2
Batch analysis for four validation batches of [18F]FE-PE2I Solution for Injection (Each batch was dispensed into vials)

Test attributes	Product Specification	PV1	PV2	PV3	Microbiological worst-case scenario (Bioburden)
Activity concentration	50–1000 MBq/mL	400 MBq/mL	600 MBq/mL	500 MBq/mL	1000 MBq/mL
Appearance	Clear or slightly yellow. Free of particles.	Clear or slightly yellow. Free of particles. (Both vials)	Clear or slightly yellow. Free of particles. (Both vials)	Clear or slightly yellow. Free of particles (Both vials)	Clear or slightly yellow. Free of particles (Both vials)
pH	4.5–8.0	6.5 (vial 1) 6.5 (vial 2)	6.5 (vial 1) 6.5 (vial 2)	6.5 (vial 1) 6.5 (vial 2)	6.5 (vial 1) 6.5 (vial 2)
Product identity [^{18}F]FE-PE2I	$[Rt_{RD} - Rt_{UV}] < 60$ sec	6 sec (vial 1) 5 sec (vial 2)	3 sec (vial 1) 2 sec (vial 2)	4 sec (vial 1) 4 sec (vial 2)	3 sec (vial 1) 4 sec (vial 2)
Chemical purity FE-PE2I	Mass limit ≤ 5 µg/patient dose	≤ 5 µg (both vials)	≤ 5 µg (both vials)	≤ 5 µg (both vials)	≤ 5 µg (both vials)
Chemical impurities	Mass limit ≤ 5 µg per patient dose	≤ 5 µg (both vials)	≤ 5 µg (both vials)	≤ 5 µg (both vials)	≤ 5 µg (both vials)
Radiochemical impurity Impurity = B [^{18}F]fluoride	≤ 5%	0% (Both vials)	1% (vial 1) 0% (vial 2)	1% (Both vials)	1% (vial 1) 0% (vial 2)
Total radiochemical purity [^{18}F]FE-PE2I $RCP_{Tot} = (100 - B) \times T$	≥ 93%	96% (Both vials)	98% (vial 1) 97% (vial 2)	97% (Both vials)	95% (vial 1) 96% (vial 2)

Test attributes	Product Specification	PV1	PV2	PV3	Microbiological worst-case scenario (Bioburden)
Mass limit	≤ 5 µg FE-PE2I and ≤ 5 µg impurities per 200 MBq (patient dose)	≤ 5 µg FE-PE2I and ≤ 5 µg impurities	≤ 5 µg FE-PE2I and ≤ 5 µg impurities	≤ 5 µg FE-PE2I and ≤ 5 µg impurities	≤ 5 µg FE-PE2I and ≤ 5 µg impurities
Residual Kryptofix 222 content	< 0.14 mg/mL	< 0.14 mg/mL (Both vials)	< 0.14 mg/mL (Both vials)	< 0.14 mg/mL (Both vials)	< 0.14 mg/mL (Both vials)
Filter integrity vial 1	≥ 3.5 bar	4.4	4.3	4.4	Bioburden
Filter integrity vial 2	≥ 3.5 bar	4.4	4.3	4.4	Bioburden
Bacterial endotoxins	< 11.5 IU/mL	< 5 EU/mL (Both vials)	< 5 EU/mL (Both vials)	< 5 EU/mL (Both vials)	< 5 EU/mL (Both vials)
Ethanol content	< 80 mg/mL	44 mg/mL (vial 1) 43 mg/mL (vial 2)	66 mg/mL (vial 1) 67 mg/mL (vial 2)	68 mg/mL (vial 1) 69 mg/mL (vial 2)	73 mg/mL (vial 1) 76 mg/mL (vial 2)
Acetonitrile	< 0.27 mg/mL	0.00 mg/mL (both vials)	0.00 mg/mL (both vials)	0.00 mg/mL (both vials)	0.00 mg/mL (vial 1) 0.09 mg/mL (vial 2)
DMSO	< 3.3 mg/mL	< 3.3 mg/mL (both vials)	< 3.3 mg/mL (both vials)	< 3.3 mg/mL (both vials)	< 3.3 mg/mL (both vials)
Sterility	Sterile, 0 CFU	Sterile (both vials)	Sterile (both vials)	Sterile (both vials)	Sterile (both vials)

Test attributes	Product Specification	PV1	PV2	PV3	Microbiological worst-case scenario (Bioburden)
Radionuclidic identity Half-life [^{68}Ga]	105–115 min	110 min (vial 1) 109 min (vial 2)	108 min (vial 1) 108 min (vial 2)	111 min (vial 1) 110 min (vial 2)	111 min (vial 1) 110 min (vial 2)
Radiochemical stability	$RCP_{Tot} \geq 93\%$ up to 6 h EOS	96% (Both vials)	96% (Both vials)	97% (Both vials)	93% (Both vials)
Shelf-life	Batch specific, calculated time where a patient dose exceeds mass limit	6 h	5 h 42 min	5 h 1 min	6 h
Abbreviations: B: percentage of radioactivity due to impurity [^{18}F]fluoride in TLC analysis; T: proportion of the radioactivity due to [^{18}F]FE-PE2I in the HPLC analysis.					

Clinical considerations

After Alzheimer´s disease, Parkinson's disorder is the second most frequent neurodegenerative condition, implying a significant impact on the quality of patients and their family's life, cost of care and work capacity. Normally, a small and slow dopaminergic reduction occurs in ordinary aging people without causing any symptoms. Dopamine deficiency in the parkinsonian brain is pronounced, emerges much faster and the symptoms are evident. Both PD and APS syndromes show decreased presynaptic neuronal degeneration. The motor symptoms develop gradually and become noticeable in the later phase of diseases when the degradation of dopaminergic neurons is about 50–80%.[16] In daily clinical practice, the diagnosis of PD and APS is usually founded on history, physical examination, and some clinical guidelines. Still there are no specific tests for certain diagnosis. Brain imaging modalities such as CT, MRI, SPECT and PET/CT may support the suspicion of Parkinson's disease and rule out other disorders.

Until recently, DaTSCAN was the only imagistic method to evaluate dopaminergic activity in the striatum at Karolinska University Hospital. In September 2022, [^{18}F]FE-PE2I was introduced, hoping for a more feasible alternative to DaTSCAN. In a comparative study between DaTSCAN and [^{18}F]FE-PE2I PET/CT, Marner et al., found a coequal sensitivity (0,94) and specificity (1.00) in both Parkinson's disease and atypical parkinsonism. [17]

The advantages of using [^{18}F]FE-PE2I PET/CT in clinical practice at Karolinska University Hospital are better spatial and temporal resolution of PET/CT compared with SPECT (Fig. 6), reduced time between tracer administration and image acquisition, reduced imaging protocol in static acquisition compared with SPECT (ca 10–15 min compared with 30 min), a more selective and detailed DAT visualization and

quantification, and no need for administration of thyroid protecting agents. Moreover, the uptake of [^{18}F]FE-PE2I is unaffected by most anti-Parkinsonian medication.

The PET/CT images in Fig. 6 represents: (A) Normal uptake of tracer in both striata in a healthy person. (B) Asymmetric tracer uptake reduction in the putaminae (arrows), with some right-side predominance, and in the right caudate nucleus, with preservation of normal uptake in the left caudate nucleus, in a patient with Parkinson's disease.

Conclusion

A fully automated synthesis of [^{18}F]FE-PE2I was developed on a commercially available radiosynthesis module, GE TRACERLab FX2 N. The decay-corrected radiochemical yield was around 39% and the radiochemical purity was greater than 95%. Overall, the protocol reliably provides a sterile and pyrogen–free GMP-compliant product suitable for clinical use in humans. [^{18}F]FE-PE2I can replace or be complement to DaTSCAN, and at the Karolinska University Hospital, about 300 patients are expected to be scanned with it yearly.

Abbreviations

- Am: Molar activity (GBq/μmol)
- APS: Atypical parkinsonians
- CNS: Central nervous system
- CT: Computed tomography
- DAT: Dopamine transporter
- DMSO: Dimethyl sulfoxide
- EOS: End of Synthesis
- FE-PE2I: (E)-N-(3-iodoprop-2-enyl)-2β-carbofluoroethoxy-3β-(4’-methyl-phenyl) nortropane
- FID: Flame ionization detector
- GC: Gas chromatography
- GE: General Electrics
- GMP: Good manufacture practice
- HPGe: High Purity Germanium Radiation detector
- HPLC: High-performance liquid chromatography
- LOD: Limit of detection
- LOQ: Limit of quantification
- MRI: Magnetic resonance imaging
- NHP: Non-Human primate

- PD: Parkinson’s disease
- PET: Positron emission tomography
- PSF: Point spread function
- QA: Quality assurance
- QP: Quality personnel
- Radio-TLC: Radio-thin layer chromatography
- RCP: Radiochemical purity (%)
- RCP_{Tot}: Total radiochemical purity
- RCY: Radiochemical yield (%)
- RD: Radio-detector
- Rt: Retention time
- SPECT: Single-photon emission computed tomography
- TOF: Time-of-flight
- UV: Ultra-violet

Declarations

Ethic approval and consent to participate

Not applicable. No animal- or clinical trials were conducted in this report.

Consent for publication

The clinical patient data used in the publication have been anonymized and safeguards measures taken to follow the GDPR (General Data Protection Regulation) requirements according to the Karolinska University Hospital’s regulation.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request. Requests for Tosylethyl-PE2I should be made to PharmaSynth.

Funding

Not applicable.

Competing interests

Not applicable.

Author’s contribution

KD, CS and TAT, were responsible for the conception, design, and coordination of the studies. MF, KD, MMM, TT, FN and TAT were responsible for the syntheses, analyses, and stability studies. MF, KD developed the radiolabelling procedure and was the major contributor to writing the manuscript. CC contributed with expertise regarding the clinical examination for which the radiotracer production is primarily intended. All authors as well as PS, KB and ES contributed to reading and approving the manuscript.

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Scheme 1

Scheme 1 is available in the Supplementary Files section.

Figures

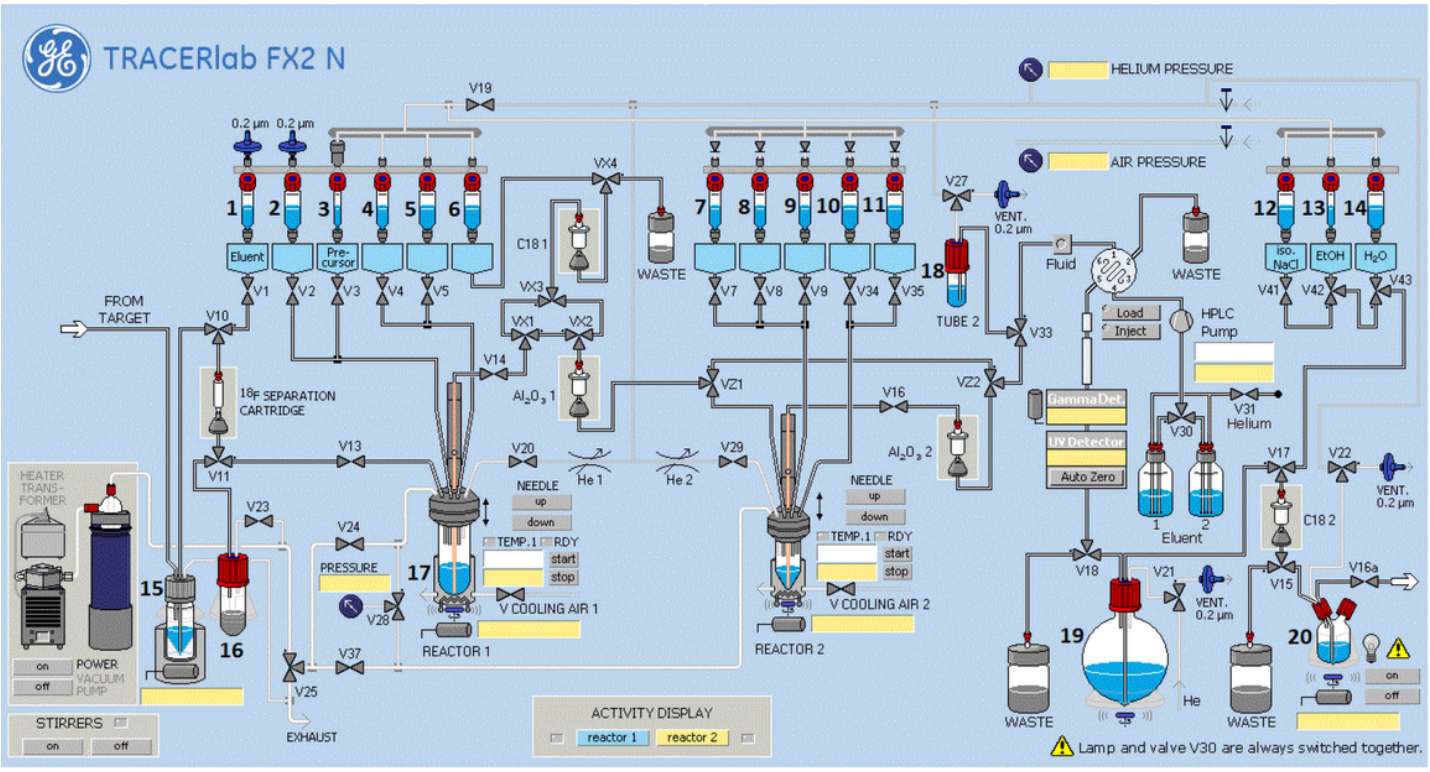

Figure 1

Schematic diagram of GE TRACERlab FX2 N module used for synthesis of [^{18}F]FE-PE2I.

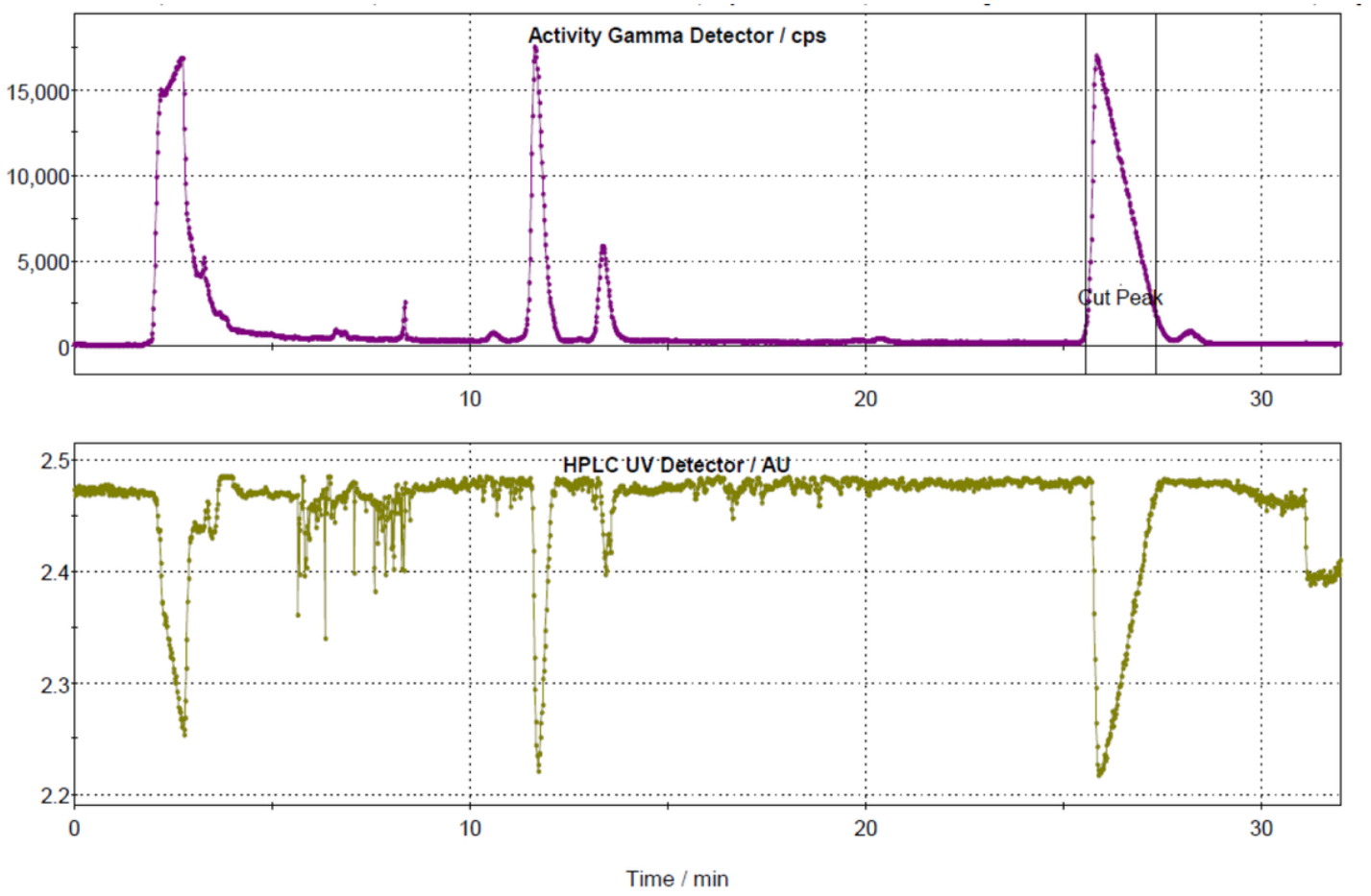

Figure 2

A typical semi-preparative HPLC chromatogram of [^{18}FE]FE-PE2I Solution for Injection.

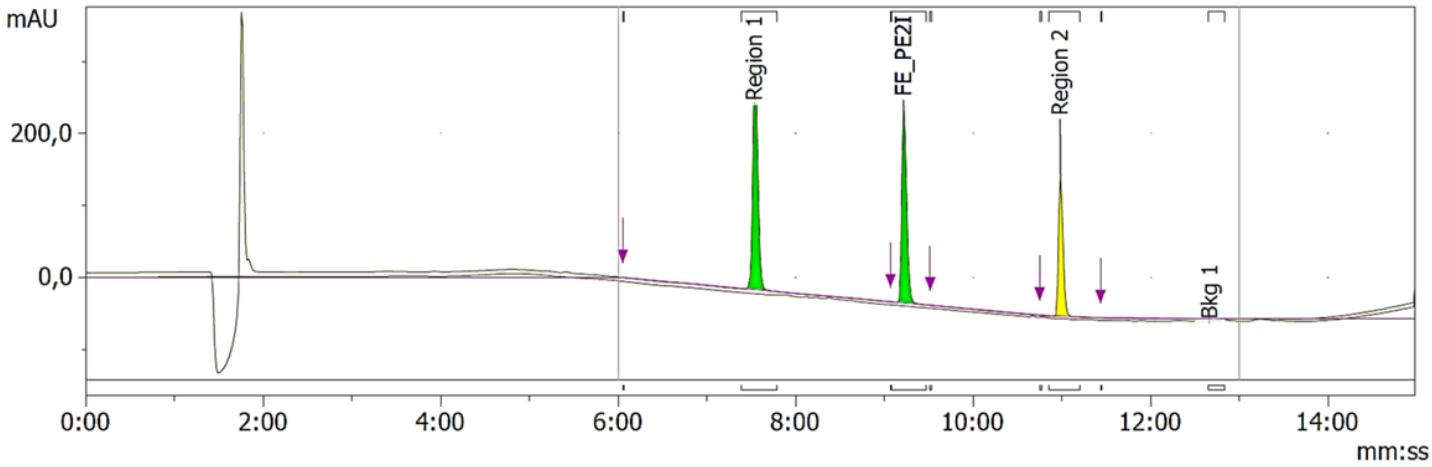

Figure 3

FE-PE2I QC SST HPLC chromatogram (5 µg/mL). Region 1: Desmethyl-PE2I, FE-PE2I and Region 2: Tosylethyl-PE2I.

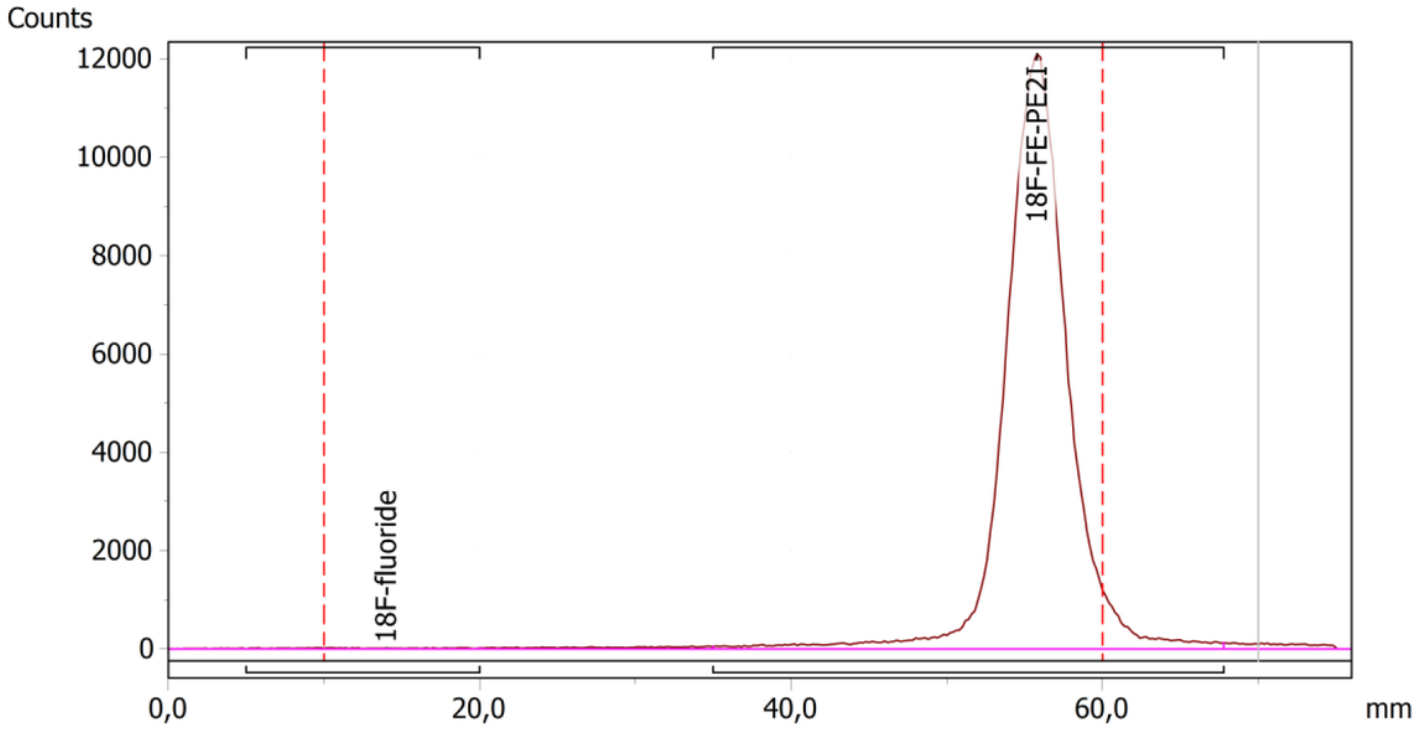

Figure 4

The TLC radio-chromatogram demonstrating the separation of [^{18}F]fluoride and [^{18}F]FE-PE2I . The retardation factor (R_f) for [^{18}F]fluoride was 0-0.1.

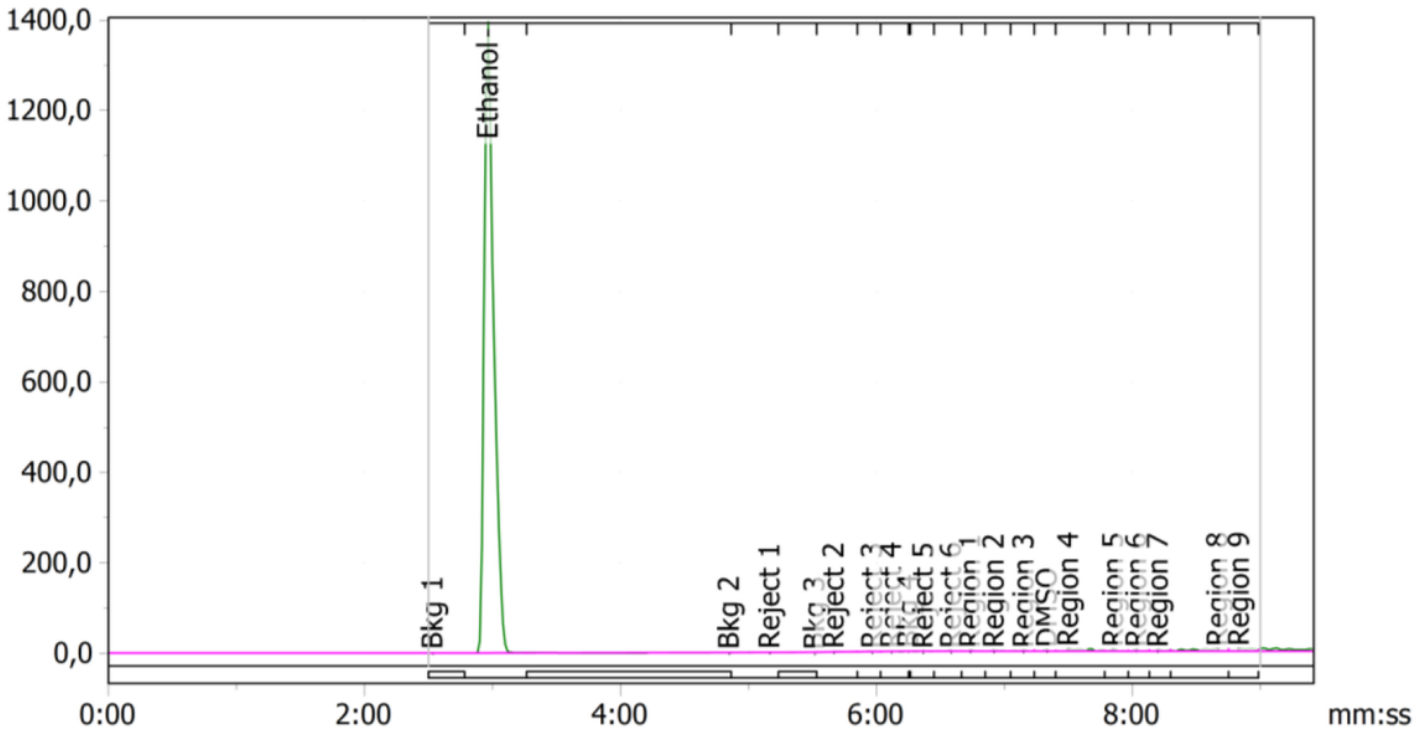

Figure 5

GC chromatogram showing a peak of ethanol (44.1 mg/mL). No traces of DMSO and acetonitrile.

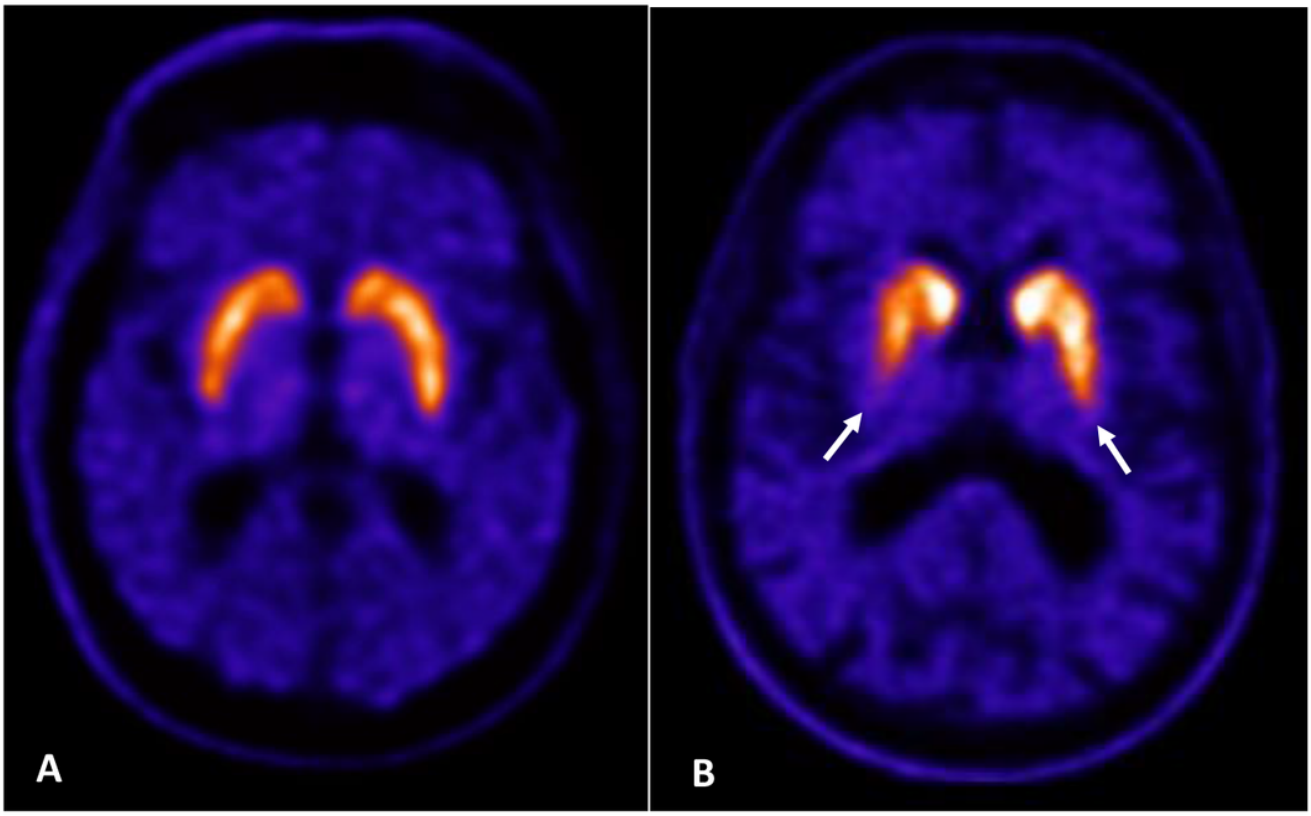

Figure 6

Representative PET/CT images with [^{18}F]FE-PE2I at the striatal level investigating Parkinson's disease and parkinsonism.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Scheme1.pdf