Journal of Visualized Experiments

Radiosynthesis of 1-(2-[18F]Fluoroethyl)-L-Tryptophan using a One-pot, Two-step Protocol

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE63025R2
Full Title:	Radiosynthesis of 1-(2-[18F]Fluoroethyl)-L-Tryptophan using a One-pot, Two-step Protocol
Corresponding Author:	Sigrid Langhans Alfred I DuPont Hospital for Children Wilmington , Delaware UNITED STATES
Corresponding Author's Institution:	Alfred I DuPont Hospital for Children
Corresponding Author E-Mail:	sigrid.langhans@nemours.org;langhans@nemoursresearch.org
Order of Authors:	Xuyi Yue
	Rahul Nikam
	Heidi Kecskemethy
	Vinay Kandula
	Stephen Falchek
	Lauren Averill
	Sigrid Langhans
Additional Information:	
Question	**Response**
Please specify the section of the submitted manuscript.	Chemistry
Please indicate whether this article will be Standard Access or Open Access.	Standard Access ($1400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Wilmington, Delaware, the United States of America
Please confirm that you have read and agree to the terms and conditions of the author license agreement that applies below:	I agree to the Author License Agreement
Please confirm that you have read and agree to the terms and conditions of the video release that applies below:	I agree to the Video Release
Please provide any comments to the journal here.	Please list Dr. Xuyi Yue as the corresponding author.

TITLE:

Radiosynthesis of 1-(2-[^{18}F]Fluoroethyl)-L-Tryptophan using a One-pot, Two-step Protocol

AUTHORS AND AFFILIATIONS:

Xuyi Yue[1,2], Rahul M. Nikam[1,2], Heidi H. Kecskemethy[1,2], Vinay V. R. Kandula[2], Stephen J. Falchek[3], Lauren W. Averill[1,2], Sigrid A. Langhans[1,4]

[1]Diagnostic & Research PET/MRI Center, Nemours/Alfred I. duPont Hospital for Children, 1600 Rockland Road, Wilmington, DE 19803, USA

[2]Department of Radiology, Nemours/Alfred I. duPont Hospital for Children, 1600 Rockland Road, Wilmington, DE 19803, USA

[3]Department of Neurology, Nemours/Alfred I. duPont Hospital for Children, 1600 Rockland Road, Wilmington, DE 19803, USA

[4]Department of Biomedical Research, Nemours/Alfred I. duPont Hospital for Children, 1600 Rockland Road, Wilmington, DE 19803, USA

Email addresses of co-authors:

Rahul Nikam (Rahul.Nikam@nemours.org)
Heidi Kecskemethy (Heidi.Kecskemethy@nemours.org)
Vinay Kandula (Vinay.Kandula@nemours.org)
Stephen Falchek (Stephen.Falchek@nemours.org)
Lauren Averill (Lauren.Averill@nemours.org)
Sigrid Langhans (Sigrid.Langhans@nemours.org)

Corresponding author:

Xuyi Yue (xuyi.yue@nemours.org)

KEYWORDS:

1-(2-[^{18}F]Fluoroethyl)-L-tryptophan, radiosynthesis, tryptophan metabolism, PET imaging, kynurenine pathway

SUMMARY:

Here, we describe the radiosynthesis of 1-(2-[^{18}F]Fluoroethyl)-L-tryptophan, a positron emission tomography imaging agent for studying tryptophan metabolism, using a one-pot, two-step strategy in a radiochemistry synthesis system with good radiochemical yields, high enantiomeric excess, and high reliability.

ABSTRACT:

The kynurenine pathway (KP) is a primary route for tryptophan metabolism. Evidence strongly suggests that metabolites of the KP play a vital role in tumor proliferation, epilepsy, neurodegenerative diseases, and psychiatric illnesses due to their immune-modulatory, neuro-modulatory, and neurotoxic effects. The most extensively used positron emission tomography (PET) agent for mapping tryptophan metabolism, α-[^{11}C]methyl-L-tryptophan ([^{11}C]AMT), has a short half-life of 20 min with laborious radiosynthesis procedures. An onsite cyclotron is required

to radiosynthesize [^{11}C]AMT. Only a limited number of centers produce [^{11}C]AMT for preclinical studies and clinical investigations. Hence, the development of an alternative imaging agent that has a longer half-life, favorable *in vivo* kinetics, and is easy to automate is urgently needed. The utility and value of 1-(2-[^{18}F]fluoroethyl)-L-tryptophan, a fluorine-18-labeled tryptophan analog, has been reported in preclinical applications in cell line-derived xenografts, patient-derived xenografts, and transgenic tumor models.

This paper presents a protocol for the radiosynthesis of 1-(2-[^{18}F]fluoroethyl)-L-tryptophan using a one-pot, two-step strategy. Using this protocol, the radiotracer can be produced in a 20 ± 5% (decay corrected at the end of synthesis, n > 20) radiochemical yield, with both radiochemical purity and enantiomeric excess of over 95%. The protocol features a small precursor amount with no more than 0.5 mL of reaction solvent in each step, low loading of potentially toxic 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (K222), and an environmentally benign and injectable mobile phase for purification. The protocol can be easily configured to produce 1-(2-[^{18}F]fluoroethyl)-L-tryptophan for clinical investigation in a commercially available module.

INTRODUCTION:

In humans, tryptophan is an essential component of the daily diet. Tryptophan is primarily metabolized via the kynurenine pathway (KP). The KP is catalyzed by two rate-limiting enzymes, indoleamine 2, 3-dioxygenase (IDO) and tryptophan 2, 3-dioxygenase (TDO). More than 95% of tryptophan is converted into kynurenine and its downstream metabolites, ultimately generating nicotinamide adenine dinucleotide, which is essential to cellular energy transduction. The KP is a key regulator of the immune system and an important regulator of neuroplasticity and neurotoxic effects[1,2]. Abnormal tryptophan metabolism is implicated in various neurologic, oncologic, psychiatric, and metabolic disorders; therefore, radiolabeled tryptophan analogs have been extensively used in clinical investigation. The two most common clinically investigated tryptophan radiotracers are ^{11}C-*α*-methyl-L-tryptophan ([^{11}C]AMT) and ^{11}C-5-hydroxytryptophan (^{11}C-5-HTP)[3].

In the 1990s, ^{11}C-5-HTP was used to visualize serotonin-secreting neuroendocrine tumors[4] and to diagnose and monitor therapy of metastatic hormone-refractory prostatic adenocarcinoma[5]. Later, it was used as an imaging tool for the quantification of the serotonergic system in the endocrine pancreas[6]. ^{11}C-5-HTP has also been a promising tracer for noninvasive detection of viable islets in intraportal islet transplantation and type 2 diabetes[7,8]. Over the past two decades, many radiolabeled amino acids have advanced to clinical investigation[9,10]. In particular, the carbon-11-labeled tryptophan analog [^{11}C]AMT has received extensive attention for mapping brain serotonin synthesis[11–14] and for localizing epileptic foci, epileptogenic tumors, tuberous sclerosis complex, gliomas, and breast cancers[15–26]. [^{11}C]AMT also has high uptake in various low- and high-grade tumors in children[27]. Furthermore, kinetic tracer analysis of [^{11}C]AMT in human subjects has been used to differentiate and grade various tumors and differentiate glioma from radiation-induced tissue injury[15]. [^{11}C]AMT-guided imaging shows significant clinical benefits in brain disorders[3,25]. However, due to the short half-life of carbon-11 (20 min) and the laborious

radiosynthesis procedures, [^{11}C]AMT use is restricted to the few PET centers with an onsite cyclotron and a radiochemistry facility.

Fluorine-18 has a favorable half-life of 109.8 min, compared with the 20 min half-life of carbon-11. Increasingly, efforts have been focused on the development of fluorine-18-labeled radiotracers for tryptophan metabolism[3,28]. A total of 15 unique fluorine-18 radiolabeled tryptophan radiotracers have been reported in terms of radiolabeling, transport mechanisms, *in vitro* and *in vivo* stability, biodistribution, and tumor update in xenografts. However, rapid *in vivo* defluorination was observed for several tracers, including 4-, 5-, and 6-[^{18}F]fluorotryptophan, precluding further clinical translation[29]. 5-[^{18}F]Fluoro-α-methyltryptophan (5-[^{18}F]FAMT) and 1-(2-[^{18}F]Fluoroethyl)-L-tryptophan (L-[^{18}F]FETrp, also known as (*S*)-2-Amino-3-(1-(2-[^{18}F]fluoroethyl)-1*H*-indol-3-yl)propanoic acid, molecular weight 249.28 g/mole), are the two most promising radiotracers with favorable *in vivo* kinetics in animal models and great potential to surpass [^{11}C]AMT for the evaluation of clinical conditions with deregulated tryptophan metabolism[28]. 5-[^{18}F]FAMT showed high uptake in IDO1-positive tumor xenografts of immunocompromised mice and is more specific to imaging the KP than [^{11}C]AMT[28,30]. However, the *in vivo* stability of 5-[^{18}F]FAMT remains a potential concern as no *in vivo* defluorination data have been reported beyond 30 min post injection of the tracer[30].

A preclinical study in a genetically engineered medulloblastoma mouse model showed that when compared with ^{18}F-fluorodeoxyglucose (^{18}F-FDG), L-[^{18}F]FETrp had high accumulation in brain tumors, negligible *in vivo* defluorination, and low background uptake, demonstrating a superior target-to-nontarget ratio[31,32]. Radiation dosimetry studies in mice indicated that L-[^{18}F]FETrp had an approximately 20% lower favorable dosimetry exposure than the clinical ^{18}F-FDG PET tracer[33]. In agreement with other researchers' findings, preclinical study data provide substantial evidence to support the clinical translation of L-[^{18}F]FETrp for the investigation of abnormal tryptophan metabolism in humans with brain disorders such as epilepsy, neuro-oncology, autism, and tuberous sclerosis[28,31–36]. An overall comparison between the three most widely investigated tracers for tryptophan metabolism, ^{11}C-5-HTP, [^{11}C]AMT, and L-[^{18}F]FETrp, is shown in **Table 1**. Both ^{11}C-5-HTP and [^{11}C]AMT have a short half-life and laborious radiolabeling procedures. A protocol for the radiosynthesis of L-[^{18}F]FETrp using a one-pot, two-step approach is described here. The protocol features the use of a small amount of radiolabeling precursor, a small volume of reaction solvents, low loading of toxic K222, and an environmentally benign and injectable mobile phase for purification and easy formulation.

PROTOCOL:

CAUTION: The protocol involves radioactive materials. Any additional dose of radioactive materials could lead to a proportional increase in the chance of adverse health effects such as cancer. Researchers must follow the 'as low as reasonably achievable' (ALARA) dose practices to guide the radiosynthesis protocol with adequate protection in the hot cell or lead hood. Minimizing direct contact time, using a lead shield, and keeping maximum distance for any radiation exposure step in the radiosynthesis process are essential. Wear a radiation dosimetry badge and hand monitoring rings throughout the entire experiment, and frequently monitor potentially contaminated surfaces such as gloves, sleeves, and feet. Nuclear Regulatory

Commission (NRC), local, and institutional regulations must be followed for the usage, shipping, and disposal of any radioactive materials.

1. Initial preparations

1.1. Prepare 10% ethanol in 50 mM sodium acetate/acetic acid mobile phase for semipreparative high-performance liquid chromatography (HPLC).

1.1.1. Place 3 mL of glacial acetic acid in a clean 1000 mL volumetric flask. Add 900 mL of ultrapure water (18 million ohm-cm resistivity at 25 °C) into the volumetric flask; add approximately 8 mL of 6 M sodium hydroxide solution and adjust the pH to 5.5 using a calibrated pH meter and a pH strip. After the solution cools down to room temperature, make up the volume to 1000 mL with ultrapure water to prepare the 50 mM sodium acetate/acetic acid (pH 5.5) solution.

1.1.2. Vacuum-filter the solution through a 0.2 µm membrane filter and transfer the solution to two 500 mL solvent bottles.

1.1.3. Place approximately 250 mL of the above buffer in a 500 mL volumetric flask. Use a graduated cylinder to measure 50 mL of United States Pharmacopeia (USP) ethanol and add the ethanol to the volumetric flask. Make up the volume to 500 mL with 50 mM sodium acetate/acetic acid, and measure the pH value with a pH strip.

1.2. Prepare quality control (QC) solutions for a system suitability test.

1.2.1. Refill the HPLC solvent bottles with fresh ultrapure water (solvent A) and ethanol (solvent B). Prime the HPLC pump and load the HPLC program with a flow rate of 1 mL/min consisting of 30% solvent A and 70% solvent B (v/v).

1.2.2. Make a control solution (blank solution) for QC. Add 5 mL of 0.9% sodium chloride into a 20 mL glass vial. Add 0.15 mL of 23.4% sodium chloride into the above solution. Add 6 mL of semipreparative HPLC mobile phase prepared in step 1.1.3 (10% ethanol in 50 mM sodium acetate/acetic acid, pH 5.5) to the glass vial.

1.2.3. Prepare 1 µg/mL of nonradiolabeled L-FETrp and 5 µg/mL of racemic L-FETrp and D-FETrp mixtures (standard solutions).

1.2.4. Build a calibration curve using standard L-FETrp solutions (0.1 µg/mL, 0.5 µg/mL, 1.0 µg/mL, 10 µg/mL, 100 µg/mL).

1.2.5. Set up the HPLC sequence. Ensure that the sequence includes one run of the blank sample solution, two runs of the standard L-FETrp (1 µg/mL), one run of the racemic L-FETrp and D-FETrp mixtures (5 µg/mL), and one run of the final radiopharmaceutical.

1.2.6. Run the partial HPLC sequence to test the system suitability before analyzing the radioactive samples using an analytical HPLC column (250 x 4.6 mm).

1.2.6.1. Run one blank sample and ensure that the chromatogram of the blank sample shows no or insignificant peaks between the void volume and 10 min of the chromatogram.

1.2.6.2. Run two replicates of the standard solution (contains 1 µg/mL of L-FETrp). Ensure that the areas of the L-FETrp in the two replicates are within ±5% of the mean value.

1.2.6.3. Run one sample each of the L-FETrp and D-FETrp mixtures (5 µg/mL of L-FETrp and D-EFTrp, respectively). Ensure that L-FETrp and D-FETrp can be identified on the chromatogram and baseline resolved.

1.3. Prepare the radiolabeling solutions and other supplies.

1.3.1. Add the following solutions to five 1.5-mL V-shaped vials, respectively. Vial 1: 1 mL of potassium carbonate (K_2CO_3)/K222 solution (5 mg/mL K222 and 1 mg/mL K_2CO_3 in a water/acetonitrile solution, 1/99, v/v) for [^{18}F]fluoride elution; Vial 2: 0.4 mL of anhydrous acetonitrile for [^{18}F]fluoride drying; Vial 3: radiolabeling precursor in anhydrous acetonitrile (1–2 mg in 0.5 mL of anhydrous acetonitrile) for [^{18}F]fluoride incorporation; Vial 4: hydrochloric acid (2 M, 0.25 mL) in acetonitrile (0.25 mL) for acidolysis; Vial 5: 2 M sodium hydroxide (0.25 mL) for neutralization of the reaction mixtures. Install the vials in the input modular vial positioner (MVP).

1.3.2. Activate a quaternary methylammonium (QMA) light cartridge by first passing through 10 mL of saturated sodium bicarbonate solution, followed by 10 mL of ultrapure water, and then flush the cartridge with nitrogen flow. Condition a light C8 cartridge and a neutral aluminum oxide cartridge by passing through 10 mL of ethanol, followed by 10 mL of ultrapure water.

1.3.3. Add 0.15 mL of 23.4% sodium chloride and 5 mL of 0.9% sodium chloride to a 30 mL sterile formulation vial to adjust the tonicity and dilute the HPLC fraction.

1.3.4. Prepare a solution (1 mL of sodium acetate/acetic acid buffer, 50 mM, pH = 5.5 prepared in step 1.1.1, 1 mL of ethanol, and 0.5 mL of water [total 2.5 mL]) in a syringe for rinsing the reaction vessel; load into a 10 mL sterile vial.

2. Assemble the radiolabeling supplies and radiosynthesize L-[^{18}F]FETrp

2.1. Assemble the radiolabeling supplies.

2.1.1. Turn on the module power, carbon dioxide, compressed air, argon lines, and the programmable logic controller (PLC) power. Click the **mod_pscf18** button to activate the program of the radiochemistry synthesis system. Initialize the input, output, and formulation MVP, and ensure that the MVPs are at positions 4, 1, 1, respectively.

2.1.2. Ensure that the HPLC loop is in the **Inject** position and the QMA light cartridge in the [^{18}F]fluoride **trapping** position.

2.1.3. Install the semipreparative HPLC mobile phase bottle (containing the solution prepared in step 1.1.3). Equilibrate the HPLC system by passing the mobile phase through the chiral HPLC column (250 x 10 mm) and C18 column (100 x 10 mm) at a flow rate of 2 mL/min for at least 30 min, then switch the diversion valve, let the HPLC mobile pass through the chiral HPLC column only, and increase the flow rate to 3 mL/min.

2.1.4. Install the QMA light cartridge in the [^{18}F]fluoride trapping/releasing line. Install the stacked alumina/C8 cartridges between the input MVP position 6 and the intermediate vial, which is a 10 mL V-shaped vial connected to the HPLC sample loop. Install a 10 mL empty vial (venting vial) to the output MVP position 4 with another needle attached to the vial as a vent. Install the reagent vials 1–5 to the input MVP positions 1–5, respectively.

2.1.5. Install the vial containing the rinse solution prepared in step 1.3.4 to the output MVP position 6.

2.1.6. Install a 500 mL waste bottle (to collect the HPLC waste passing through both the chiral and C18 columns) to the formulation MVP position 1. Install another 500 mL waste bottle (to collect HPLC waste passing through the chiral column) to the waste collection end of the four-port two-position valve.

2.1.7. Connect the fraction collection vial (prefilled solution prepared in step 1.3.3) to the formulation MVP position 2. Connect the output MVP position 3 (gas line) and final product delivery line to the formulation MVP position 2 vials to recover the final formulated product. Install a 10 mL empty sterile vial in formulation MVP position 3, which will be used as the backup vial for the target fraction collection.

2.1.8. Install the C18 short column between the four-port, two-position diversion valve and the formulation MVP.

NOTE: During the installation of the reagent and formulation vials, ensure the argon supply in the control panel is off, and the argon pressure is zero to avoid any unexpected liquid transfer during the vial assembly. Double-check all needle connections, vial positions, and MVP positions for reproducible radiosynthesis.

2.2. Radiosynthesis of L-[^{18}F]FETrp

2.2.1. Receive and survey [^{18}F]fluoride.

2.2.1.1. When receiving the [^{18}F]fluoride solution (15 ± 3 gigabecquerel (GBq) at the start of synthesis, see the **Table of Materials**), survey the lead box on the surface and at 1 m to record the maximum radiation exposure rates. Do a wipe test to ensure the shipping box is not

contaminated. Record the [^{18}F]fluoride dose and time.

2.2.2. Transfer [^{18}F]fluoride.

2.2.2.1. Transfer the radioactivity to the radiochemistry synthesis system.

2.2.2.2. Connect the argon line with a short needle and the [^{18}F]fluoride transfer line with a long needle to the [^{18}F]fluoride vial. Close the hot-cell glass door and lead door.

CAUTION: Use a long clamp to push down both needles; ensure the long needle tip sits in the bottom of the [^{18}F]fluoride vial so that all [^{18}F]fluoride can be transferred out. Typically, a V-shaped vial is requested for the [^{18}F]fluoride delivery.

2.2.3. Trap, elute, and azeotropically dry [^{18}F]fluoride.

2.2.3.1. Click **Ar Supply** to turn on the argon supply line, increase argon pressure, turn on the [^{18}F]fluoride pushing line, and push the aqueous [^{18}F]fluoride through the QMA light cartridge. After all the radioactivity is trapped in the QMA light cartridge, and the radioactivity detector reading is steady, increase the argon pressure and blow argon through the cartridge for another 5 min to remove excess water.

2.2.3.2. Turn off the [^{18}F]fluoride pushing line, decrease the argon pressure to zero, switch the six-port two-position valve from the [^{18}F]fluoride trapping position to the elution position. Open the reaction vial, turn on the input MVP position 1 argon line, push the K222/K_2CO_3 solution into the input MVP position 1 vial through the QMA light cartridge to elute out the radioactivity into the reaction vial. Switch the [^{18}F]fluoride elution position to the trapping position.

2.2.3.3. Click the **Heat** button to heat the reactor at 110 °C, turn on the output MVP position 4 argon line (sweeping line) that connects to the reactor, and evaporate the solvent into the output MVP position 4 vial.

2.2.3.4. Click the **Cool** button to cool down the reactor to room temperature with compressed carbon dioxide, turn off the sweeping line, switch the input MVP position 1 to position 2, and add the anhydrous acetonitrile in the vial 2. Turn on the sweeping line and heater to azeotropically dry [^{18}F]fluoride at 110 °C.

2.2.4. Add the radiolabeling precursor and incorporate [^{18}F]fluoride.

2.2.4.1. Cool down the reactor to room temperature, turn off the sweeping line, switch the input MVP position 2 to position 3, and add the tosylate radiolabeling precursor in vial 3. Close the reactor, and heat the reaction mixtures at 100 °C for 10 min.

2.2.5. Evaporate the reaction solvent and acidolyse.

2.2.5.1. Cool down and open the reactor, turn on the sweeping line, and evaporate the reaction solvent at 100 °C.

2.2.5.2. Cool down the reactor, turn off the sweeping line, switch the input MVP position 3 to position 4, and add the hydrochloric acid/acetonitrile mixture (0.5 mL, 1/1, v/v). Heat the reaction at 100 °C for 10 min to deprotect the *tert*-butyl and *tert*-butyloxycarbonyl-protecting groups in the radiolabeling precursor.

2.2.6. Neutralize the reaction.

2.2.6.1. Cool down the reactor to room temperature, and switch the input MVP position 4 to position 5 to add 2 M sodium hydroxide to neutralize the reaction mixture. Turn off the input MVP argon line.

2.2.7. Transfer the reaction mixture to the intermediate vial.

2.2.7.1. Click the **F** button in the output MVP to switch the output MVP from the venting position 4 to position 5, and then click the **F** button in the input MVP to switch the input MVP from position 5 to position 6. Turn on the output MVP argon line. Push the reaction mixture through the stacked neutral aluminum oxide and C8 cartridges to the intermediate vial installed before the HPLC sample loop.

2.2.8. Rinse the reactor and transfer the solution.

2.2.8.1. Switch the output MVP position 5 to position 6, push the rinse solution in the vial of output MVP position 6 through the reaction vial, and cartridges to the intermediate vial, successively. Note that the volume of the combined mixture is approximately 3.5 mL. Close the reaction vessel.

2.2.9. Load the combined mixture to the HPLC loop and purify the mixture.

2.2.9.1. Switch the HPLC loop from injection position to load position, turn on the input MVP argon line, and load the mixtures to the 5 mL HPLC loop. When the load is complete, switch the load button to inject, and click **Inject HPLC** to start the HPLC chromatogram at a flow rate of 3 mL/min. Click the **HPLC monitor** button to access the real-time HPLC chromatogram.

2.2.10. Divert the target fraction to the C18 column.

2.2.10.1. Click the **diversion MVP** button to divert the target HPLC fraction to the short C18 column at approximately 12 min.

2.2.11. Flush the chiral HPLC column and purify the fraction in the C18 column.

2.2.11.1. After the target radioactivity is collected in the C18 column (and the HPLC mobile

phase flows into the formulation MVP position 1 waste bottle), switch the four-port two-position diversion valve to the waste collection position. Flush the chiral column at 4 mL/min for 6 min to remove the minor D-[^{18}F]FETrp enantiomer and other ultraviolet (UV) impurities.

2.2.11.2. Click the **diversion MVP** button to divert the HPLC mobile phase back to the formulation MVP position 1 at a flow rate of 3 mL/min. Observe that the mobile phase passes through the chiral column and C18 column to purify L-[^{18}F]FETrp retained in the C18 column.

2.2.12. Collect the target fraction.

2.2.12.1. Collect the eluent at approximately 32–34 min into the formulation MVP position 2 vial.

NOTE: Typically, the 2 min HPLC fraction is collected, and the total volume plus the prefilled sodium chloride solution is 8–15 mL.

2.2.13. Deliver the dose to a sterile final product vial.

2.2.13.1. Push the solution in the formulation MVP position 2 vial through the delivery line and sterile filter into the final dose vial (via the preinstalled sterile venting filter needle).

NOTE: Protocol steps 2.2.9–2.2.13 are steps used for the HPLC purification of L-[^{18}F]FETrp.

2.2.14. Assay the radioactivity and dose volume.

2.2.14.1. Remove the sterile filter and assay the final dose activity and volume.

2.2.14.2. Flush the system with ultrapure water followed by ethanol at 4 mL/min for at least 15 min each. Turn off the HPLC pump and PLC box; close the program; shut down the compressed air line, argon line, and carbon dioxide line; and shut down the main power of the module.

2.2.15. Withdraw the QC dose and run the QC samples.

2.2.15.1. Withdraw approximately 0.1 mL of the final dose into a 0.2 mL insert of a QC vial. Run the hot sample as the “partial sequence” program following the system suitability test described in step 1.2.6**.**

2.2.16. Analyze the QC data and release the dose.

2.2.16.1. Calculate the chemical and radiochemical purities, enantiomeric excess value, and molar activity; determine the pH value.

2.2.16.2. Release the dose if all testing results pass the acceptable range.

3. Post-run system clean

3.1. Survey the radiolabeling module using a Geiger-Mueller (GM) survey meter to ensure that the radioactivity is adequately decayed (at least 24 h) before the system clean.

3.2. Turn on the radiolabeling module power and PLC box power; activate the program of the radiochemistry synthesis system; and initialize the input MVP, output MVP, and formulation MVP. Switch the column selector to the bypass position.

3.3. Detach the QMA light, alumina, and C8 cartridges.

3.4. Flush all vials and lines with ultrapure water first, followed by ethanol. Dry the vials and lines with high-purity argon.

3.5. Seal each line vent using a sterile needle with a cap. Replace the reagent vials and reaction vessel with oven-burned vials and reaction vessel, respectively.

3.6. Turn off the HPLC pump and PLC box; close the program; shut down the compressed air line, argon line, and carbon dioxide line; and shut down the main power of the module.

REPRESENTATIVE RESULTS:

The reaction scheme is shown in **Figure 1**. The radiolabeling includes the following two steps: 1) reaction of the tosylate radiolabeling precursor with [^{18}F]fluoride provides the ^{18}F-labeled intermediate, and 2) deprotection of the *tert*-butyloxycarbonyl and *tert*-butyl-protecting groups in the intermediate affords the final product L-[^{18}F]FETrp. Both reaction steps continue at 100 °C for 10 min.

Before receiving [^{18}F]fluoride from the commercial vendor, assemble the reagent vials, formulation vials, and cartridges; equilibrate the semi-preparative, QC systems; and run a QC system suitability test. The detailed workflow for the radiosynthesis of L-[^{18}F]FETrp is outlined in **Figure 2**. In brief, the radioactivity is surveyed and transferred to the radiochemistry synthesis system, and the [^{18}F]fluoride is azeotropically dried in the reaction vessel after the trapping/releasing steps. After the [^{18}F]fluoride incorporation in the first step, acid is added to deprotect the two functional groups, followed by base neutralization. The reaction mixture is transferred to an intermediate vial, and the reaction vessel is rinsed with a mixed solution. The combined mixture is loaded onto the HPLC loop for purification. A combination of chiral and C18 HPLC columns is used to remove the chemical impurities. The target fraction is collected into a formulation vial prefilled with sodium chloride to adjust the dose concentration and tonicity. The final product is sterile-filtered into a final-dose vial, assayed, and aliquoted for QC before the doses are released.

The schematic diagram of the system setup is shown in **Figure 3**. The module consists of the following major components: 1) input MVP for reagent addition, 2) output MVP for reactor venting and rinse, 3) formulation MVP for HPLC fraction collection and dose formulation, 4)

[^{18}F]fluoride trapping and releasing MVP, and 5) HPLC purification system. The trends for radioactivity, reaction temperature, and pressure can be monitored in real time through the control panel. A typical semipreparative HPLC chromatogram is shown in **Figure 4**. The target fraction containing UV impurities is diverted to a short C18 column (the black trace overlapped with the red UV trace, **Figure 4**). The impurities in the target component can be removed by passing the fraction through a C18 column. The purified HPLC fraction eluted from the C18 column is collected in the formulation vial. The dose is assayed and aliquoted for QC.

The representative HPLC chromatograms for QC are shown in **Figure 5**. The chromatogram of the blank sample shows insignificant peaks between the void volume and 10 min of the program. The nonradiolabeled standard reference L-FETrp shows a single isomer, separated well from the standard reference of its D-counterpart. The final dose of L-[^{18}F]FETrp shows high chemical purity and radiochemical purity. Stability testing of the final product at the highest dose concentration for up to 8 h shows that L-[^{18}F]FETrp is stable in terms of chemical purity, radiochemical purity, enantiomeric excess, and pH value (**Table 2**)[37]. This protocol for the one-pot, two-step radiosynthesis of L-[^{18}F]FET takes approximately 100 min. The decay-corrected yield is 20 ± 5%, with chemical and radiochemical purities greater than 95%. Starting from 12–18 GBq of [^{18}F]fluoride, the molarity activity of L-[^{18}F]FET is 88–118 GBq/μmol. The mass concentration is typically less than 0.5 μg/mL, with the dose concentration in the range of 37–185 MBq/mL.

FIGURE AND TABLE LEGENDS:

Figure 1: Reaction scheme for one-pot, two-step radiosynthesis of L-[^{18}F]FETrp. Abbreviations: MeCN = acetonitrile; L-[^{18}F]FETrp = 1-(2-[^{18}F]Fluoroethyl)-L-tryptophan; K222 = 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane.

Figure 2: Overview of the L-[^{18}F]FETrp radiosynthesis workflow. *A complete quality control according to USP823, USP797 will be followed for human use of L-[^{18}F]FETrp. Abbreviations: QC = quality control; MeCN = acetonitrile; L-[^{18}F]FETrp = 1-(2-[^{18}F]Fluoroethyl)-L-tryptophan.

Figure 3: Production of L-[^{18}F]FETrp. Schematic diagram of setup (left) and the photograph of the radiosynthesis platform (right). The setup includes the following major components: 1. Input MVP; 2. Output MVP; 3. Formulation MVP; 4. [^{18}F]Fluoride-trapping/releasing MVP; 5. QMA cartridge; 6. Intermediate vial; 7. Alumina/C8 cartridges; 8. Reactor; 9. Chiral HPLC column; 10, C18 column; 11. HPLC waste bottle to the chiral column; 12. Diversion MVP; 13. HPLC waste bottle to the chiral and C18 columns; 14. Formulation vial; 15. Back up vial. Abbreviations: L-[^{18}F]FETrp = 1-(2-[^{18}F]Fluoroethyl)-L-tryptophan; MVP = modular vial positioner; QMA = quaternary methylammonium.

Figure 4: **Typical semipreparative chromatogram for purification of L-[^{18}F]FETrp.** Red trace, UV channel at 254 nm. Black trace, radioactivity channel. Arrows 1, 2 indicate the start and end of diverting the radioactive fraction containing L-[^{18}F]FETrp to the C18 column, respectively. Arrows 3, 4 indicate the start and end of collecting the purified target fraction L-[^{18}F]FETrp eluted from the C18 column, respectively. Abbreviation: L-[^{18}F]FETrp = 1-(2-[^{18}F]Fluoroethyl)-L-tryptophan.

Figure 5: **Typical analytical HPLC chromatogram for quality control of L-[^{18}F]FETrp.** 1) Blank solution, 2) standard solution of L-FETrp, 3) standard solution of L-FETrp and D-FETrp mixtures, 4) UV trace of L-FETrp at 230 nm, 4) radioactivity trace of L-[^{18}F]FETrp formulation. Abbreviation: L-[^{18}F]FETrp = 1-(2-[^{18}F]Fluoroethyl)-L-tryptophan; L-FETrp = 1-(2-Fluoroethyl)-L-tryptophan; D-FETrp = 1-(2-Fluoroethyl)-D-tryptophan.

Table 1: **Comparison of ^{11}C-5-HTP, [^{11}C]AMT, and L-[^{18}F]FETrp.** Abbreviations: cGMP = current good manufacturing practices; α-[^{11}C]methyl-L-tryptophan; L-[^{18}F]FETrp = 1-(2-[^{18}F]Fluoroethyl)-L-tryptophan; ^{11}C-5-HTP = ^{11}C-5-hydroxytryptophan.

Table 2: **Stability test of L-[^{18}F]FETrp in a typical batch at the highest dose concentration.** Abbreviation: L-[^{18}F]FETrp = 1-(2-[^{18}F]Fluoroethyl)-L-tryptophan.

DISCUSSION:

Tryptophan is an essential amino acid for humans. It plays an important role in the regulation of mood, cognitive function, and behavior. Radiolabeled tryptophan derivatives, particularly the carbon-11-labeled [^{11}C]AMT, have been extensively studied due to their unique role in mapping serotonin synthesis[38,39], detecting and grading tumors[40], guiding epilepsy surgery[41,42], and evaluating treatment response in diabetes[43]. However, the short half-life and laborious radiolabeling procedures limit the widespread application of [^{11}C]AMT. Efforts are underway to develop fluorine-18-labeled agents for tryptophan metabolism. Two recent review articles summarize the development and imaging properties of fluorine-18-labeled tryptophan imaging agents[3,28].

Compared with its ^{11}C-labeled predecessor, L-[^{18}F]FETrp demonstrates favorable *in vivo* imaging properties, good metabolic stability, and resistance to defluorination[33]. Additionally, L-[^{18}F]FETrp demonstrates a favorable dosimetry profile compared to ^{18}F-FDG and has been proposed as a promising tryptophan imaging agent for clinical translation[32,33]. The methodology described here utilizes a one-pot, two-step strategy for the radiosynthesis of L-[^{18}F]FETrp in a radiochemistry synthesis system. L-[^{18}F]FETrp was produced with high chemical purity, radiochemical purity, and enantiomeric excess. The total nonradiolabeled L-FETrp mass in the final dose is no more than 5 µg, and the ethanol content is no more than 10%. L-[^{18}F]FETrp is routinely produced in the PET center for the imaging of tryptophan metabolism in a transgenic medulloblastoma mouse brain tumor model and has shown favorable imaging results[32]. When compared with the reported method for L-[^{18}F]FETrp, the current protocol includes the benefits detailed below.

First, a small reaction vessel and less precursor and reaction solvents are used for the radiolabeling when compared with other reported radiolabeling modules and methods (in which 9 of mg precursor in 1.1 mL of solvent was used)[35], and only 1–2 mg of radiolabeling precursor in 0.5 mL of solvent is added to the reaction but with a much higher yield of the enantiomer. Less than 1% yield has been reported for a two-pot, three-step radiosynthesis of L-[^{18}F]FETrp without any report of the enantiomeric excess value[44].

Second, the lowest amount of toxic K222, compared with reported procedures for L-[^{18}F]FETrp or racemic [^{18}F]FETrp, is used. Typically 4–5 mg of K222 is used compared with 37.5 mg used by others[35]. K222 is a phase transfer catalyst frequently used in the radiosynthesis of ^{18}F-labeled PET tracers. The limit specified in the USP for K222 is less than 50 μg/mL. A color spot test for the detection of the residual K222 concentration must be performed to meet the criteria before releasing the final dose for clinical use[45].

Third, only 1% water is introduced to the K_2CO_3/K222 solution for [^{18}F]fluoride elution, which expedites the drying process of aqueous [^{18}F]fluoride. [^{18}F]fluoride anions are heavily hydrated and become chemically inert in aqueous media[46]. Therefore, enhancing the nucleophilicity by desolvating [^{18}F]fluoride and azeotropic drying of the aqueous solution is required for [^{18}F]fluoride incorporation. Water will also compete with [^{18}F]fluoride to hydrolyze instead of the desired [^{18}F]fluoride nucleophilic substitution of the radiolabeling precursor.

Fourth, an injectable mobile phase is used for the purification of L-[^{18}F]FETrp. Ten percent ethanol in 50 mM sodium acetate/acetic acid, pH 5.5, is used as the mobile phase to purify the radiotracer, readily bringing the ethanol content to less than 10% in the final dose for clinical use. While 90% ethanol in water has been reported to resolve the enantiomers, it takes more time to evaporate the ethanol content to less than 10% at 78 °C[34].

The preclinical study of L-[^{18}F]FETrp in a transgenic medulloblastoma mouse model shows 1-L-[^{18}F]FETrp had high brain tumor accumulation with favorable kinetics, negligible *in vivo* defluorination, and low background uptake[32]. 1-L-[^{18}F]FETrp also shows a superior target-to-nontarget ratio to ^{18}F-FDG[31]. Furthermore, the protocol is easy to set up for the production of L-[^{18}F]FETrp for clinical investigation[37]. Additional, comprehensive QC tests, including filter integrity, radionuclidic purity, residual solvent levels, K222 concentration, bacterial endotoxin level, and sterility tests, can be readily performed for the final dose of the radiopharmaceutical. The process of regulatory approval for the clinical utilization of L-[^{18}F]FETrp in human subjects is actively ongoing.

The method has some limitations. Two HPLC columns are used to obtain adequate chemical purity and enantiomeric excess of L-[^{18}F]FETrp. A flow rate of the mobile phase at 3 mL/min is used for the purification. A higher flow rate results in high backpressure, while a lower flow rate leads to extended time for purification and poor baseline resolution of the peaks. Alternative HPLC columns that are compatible with the mobile phase and show better selectivity towards enantiomers and good resolution over the impurities may simplify the purification steps.

The radiochemistry synthesis module is a noncommercial system. The fully automated radiosynthesis of racemic [^{18}F]FETrp has been reported in a commercial GE FASTlab synthesizer. Chiral separation of the enantiomers is performed with a chiral analytical HPLC column; the final L- and D-isomers are formulated on a second FASTLab cassette[35]. Xin and Cai[34] reported the automatic radiosynthesis of optically pure L-[^{18}F]FETrp using a GE FX-N system. While the two enantiomers can be readily separated with the semipreparative chiral HPLC column, the mobile phase with a high ethanol content (90% ethanol in water) is not suitable for direct human

injection[34]. The use of a commercial radiosynthesizer and an injectable mobile phase for L-[^{18}F]FETrp with high enantiomeric excess is highly desirable for easy clinical investigation.

In conclusion, a fluorine-18-labeled tryptophan analog L-[^{18}F]FETrp was synthesized in a radiochemistry synthesis system using a one-pot, two-step approach with high reliability and reproducibility. The radiosynthesis features small amounts of radiolabeling precursor and solvents, an injectable mobile phase, and easy implementation for clinical production of L-[^{18}F]FETrp for human use. The protocol will facilitate more widespread utilization of this radiotracer for neurological disorders and cancers implicated with tryptophan metabolism.

ACKNOWLEDGMENTS:

This work was supported by the Diagnostic & Research PET/MRI Center, and by the Departments of Biomedical Research and Radiology at Nemours/Alfred duPont Hospital for Children.

DISCLOSURES:

The authors declare that no competing financial interests exist.

REFERENCES:

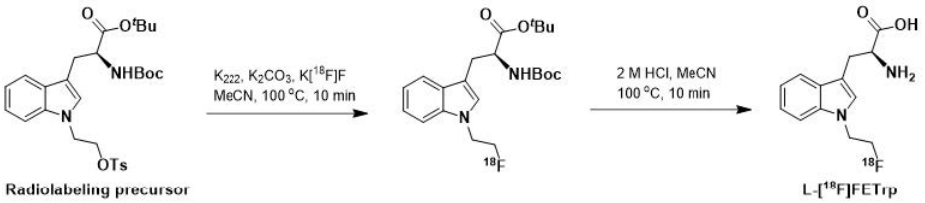
1. Cetina Biefer, H. R., Vasudevan, A., Elkhal, A. Aspects of tryptophan and nicotinamide adenine dinucleotide in immunity: A new twist in an old tale. *International Journal of Tryptophan Research*. **10**, 1178646917713491 (2017).
2. Savitz, J. The kynurenine pathway: a finger in every pie. *Molecular Psychiatry*. **25** (1), 131–147 (2020).
3. Zlatopolskiy, B. D. et al. 11C- and 18F-labelled tryptophans as PET-tracers for imaging of altered tryptophan metabolism in age-associated disorders. *Russian Chemical Reviews*. **89** (9), 879–896 (2020).
4. Eriksson, B. et al. Positron emission tomography (PET) in neuroendocrine gastrointestinal tumors. *Acta Oncologica*. **32** (2), 189–196 (1993).
5. Kälkner, K. M. et al. Positron emission tomography (PET) with ^{11}C-5-Hydroxytryptophan (5-HTP) in patients with metastatic hormone-refractory prostatic adenocarcinoma. *Nuclear Medicine and Biology*. **24** (4), 319–325 (1997).
6. Eriksson, O. et al. Quantitative imaging of serotonergic biosynthesis and degradation in the endocrine pancreas. *Journal of Nuclear Medicine*. **55** (3), 460–465 (2014).
7. Carlbom, L. et al. [^{11}C]5-hydroxy-tryptophan pet for assessment of islet mass during progression of type 2 diabetes. *Diabetes*. **66** (5), 1286–1292 (2017).
8. Eriksson, O. et al. Positron emission tomography to assess the outcome of intraportal islet transplantation. *Diabetes*. **65** (9), 2482–2489 (2016).
9. Jager, P. L. et al. Radiolabeled amino acids: Basic aspects and clinical applications in oncology. *Journal of Nuclear Medicine*. **42** (3), 432–445 (2001).
10. Langen, K. J., Galldiks, N. Update on amino acid pet of brain tumours. *Current Opinion in Neurology*. **31** (4), 354–361 (2018).
11. Chugani, D. C., Muzik, O., Chakraborty, P., Mangner, T., Chugani, H. T. Human brain serotonin synthesis capacity measured in vivo with α-[C-11]methyl-L-tryptophan. *Synapse*. **28** (1), 33–43 (1998).

12. Chugani, D. C., Muzik, O. Alpha[C-11]methyl-L-tryptophan PET maps brain serotonin synthesis and Kynurenine pathway metabolism. *Journal of Cerebral Blood Flow and Metabolism*. **20**, 2–9 (2000).
13. Diksic, M., Nagahiro, S., Sourkes, T. L., Yamamoto, Y. L. A new method to measure brain serotonin synthesis in vivo. I. Theory and basic data for a biological model. *Journal of Cerebral Blood Flow and Metabolism*. **10** (1), 1–12 (1990).
14. Diksic, M., Young, S. N. Study of the brain serotonergic system with labeled α-methyl-L-tryptophan. *Journal of Neurochemistry*. **78** (6), 1185–1200 (2001).
15. Alkonyi, B. et al. Accurate differentiation of recurrent gliomas from radiation injury by kinetic analysis of α-^{11}C-methyl-L-tryptophan PET. *Journal of Nuclear Medicine*. **53**, 1058–1064 (2012).
16. Bagla, S. et al. A distinct microRNA expression profile is associated with α[^{11}C]-methyl-L-tryptophan (AMT) PET uptake in epileptogenic cortical tubers resected from patients with tuberous sclerosis complex. *Neurobiology of Disease*. **109**, 76–87 (2018).
17. Alkonyi, B. et al. Increased tryptophan transport in epileptogenic dysembryoplastic neuroepithelial tumors. *Journal of Neuro-oncology*. **107** (2), 365–372 (2012).
18. Chugani, D. C. α-methyl-L-tryptophan: Mechanisms for tracer localization of epileptogenic brain regions. *Biomarkers in Medicine*. **5** (5), 567–575 (2011).
19. Chugani, D. C. et al. Imaging epileptogenic tubers in children with tuberous sclerosis complex using α-[11C]methyl-L-tryptophan positron emission tomography. *Annals of Neurology*. **44** (6), 858–866 (1998).
20. Chugani, H. T. et al. α-[^{11}C]-Methyl-L-tryptophan-PET in 191 patients with tuberous sclerosis complex. *Neurology*. **81** (7), 674–680 (2013).
21. Jeong, J. W. et al. Multi-modal imaging of tumor cellularity and tryptophan metabolism in human Gliomas. *Cancer Imaging*. **15** (1), 10 (2015).
22. Juhász, C. et al. Quantitative PET imaging of tryptophan accumulation in gliomas and remote cortex. *Clinical Nuclear Medicine*. **37** (9), 838–842 (2012).
23. Juhász, C. et al. Tryptophan metabolism in breast cancers: Molecular imaging and immunohistochemistry studies. *Nuclear Medicine and Biology*. **39** (7), 926–932 (2012).
24. Juhász, C. et al. Successful surgical treatment of an inflammatory lesion associated with new-onset refractory status epilepticus. *Neurosurgical Focus*. **34**, E5 (2013).
25. Kumar, A., Asano, E., Chugani, H.T. α-[^{11}C]-methyl-L-tryptophan PET for tracer localization of epileptogenic brain regions: Clinical studies. *Biomarkers in Medicine*. **5** (5), 577–584 (2011).
26. Tiwari, V. N., Kumar, A., Chakraborty, P. K., Chugani, H. T. Can diffusion tensor imaging (DTI) identify epileptogenic tubers in tuberous sclerosis complex? Correlation with α-[11C]methyl-L-tryptophan ([11C]AMT) positron emission tomography (PET). *Journal of Child Neurology*. **27** (5), 598–603 (2012).
27. Juhász, C. et al. In vivo uptake and metabolism of α-[^{11}C]methyl-L-tryptophan in human brain tumors. *Journal of Cerebral Blood Flow and Metabolism*. **26** (3), 345–357 (2006).
28. John, F., Muzik, O., Mittal, S., Juhász, C. Fluorine-18-labeled PET radiotracers for imaging tryptophan uptake and metabolism: a systematic review. *Molecular Imaging and Biology*. **22** (4), 805–819 (2020).
29. Zlatopolskiy, B. D. et al. Discovery of 7-[18 F]fluorotryptophan as a novel positron emission tomography (PET) probe for the visualization of tryptophan metabolism in vivo. *Journal*

660 *of Medicinal Chemistry*. **61** (1), 189–206 (2018).
661 30. Giglio, B. C. et al. Synthesis of 5-[^{18}F]fluoro-α-methyl tryptophan: New trp based PET
662 agents. *Theranostics*. **7** (6), 1524–1530 (2017).
663 31. Yue, X. et al. Comparison of 1-(2-[^{18}F]fluoroethyl)-L-tryptophan and FDG for the detection
664 of medulloblastoma in a transgenic mouse model. *Journal of Nuclear Medicine*. **60** (Supplement
665 1), 545 (2019).
666 32. Xin, Y. et al. PET imaging of medulloblastoma with an 18F-labeled tryptophan analogue in
667 a transgenic mouse model. *Scientific Reports*. **10** (1), 3800 (2020).
668 33. Michelhaugh, S. K. et al. Assessment of tryptophan uptake and kinetics using 1-(2-18F-
669 fluoroethyl)-L-tryptophan and α-^{11}C-methyl-L-tryptophan PET imaging in mice implanted with
670 patient-derived brain tumor xenografts. *Journal of Nuclear Medicine*. **58** (2), 208–213 (2017).
671 34. Xin, Y., Cai, H. Improved radiosynthesis and biological evaluations of L- and D-1-
672 [^{18}F]fluoroethyl-tryptophan for PET imaging of IDO-mediated kynurenine pathway of tryptophan
673 metabolism. *Molecular Imaging and Biology*. **19** (4), 589–598 (2017).
674 35. Henrottin, J. et al. Fully automated radiosynthesis of N1-[^{18}F]fluoroethyl-tryptophan and
675 study of its biological activity as a new potential substrate for indoleamine 2,3-dioxygenase PET
676 imaging. *Nuclear Medicine and Biology*. **43** (6), 379–389 (2016).
677 36. Xin, Y. et al. Evaluation of l-1-[^{18}F]Fluoroethyl-tryptophan for PET imaging of cancer.
678 *Molecular Imaging and Biology*. **21** (6), 1138–1146 (2019).
679 37. Yue, X. et al. Automated production of 1-(2-[^{18}F]fluoroethyl)-L-tryptophan for imaging of
680 tryptophan metabolism. *Applied Radiation and Isotopes*. **156**, 109022 (2020).
681 38. Booij, L. et al. Brain serotonin synthesis in adult males characterized by physical
682 aggression during childhood: A 21-year longitudinal study. *PLoS ONE*. **5** (6), e11255(2010).
683 39. Chandana, S. R. et al. Significance of abnormalities in developmental trajectory and
684 asymmetry of cortical serotonin synthesis in autism. *International Journal of Developmental*
685 *Neuroscience*. **23** (2–3), 171–182 (2005).
686 40. Juhász, C., Dwivedi, S., Kamson, D. O., Michelhaugh, S. K., Mittal, S. Comparison of amino
687 acid positron emission tomographic radiotracers for molecular imaging of primary and metastatic
688 brain tumors. *Molecular Imaging*. **13** (6), 1–10 (2014).
689 41. Rubí, S. et al. Positron emission tomography with α-[^{11}C]methyl-L-tryptophan in tuberous
690 sclerosis complex-related epilepsy. *Epilepsia*. **54** (12), 2143–2150 (2013).
691 42. Chugani, H. T. et al. Clinical and histopathologic correlates of 11C-alpha-methyl-L-
692 tryptophan (AMT) PET abnormalities in children with intractable epilepsy. *Epilepsia*. **52** (9), 1692–
693 1698 (2011).
694 43. Muzik, O., Burghardt, P., Yi, Z., Kumar, A., Seyoum, B. Successful metformin treatment of
695 insulin resistance is associated with down-regulation of the kynurenine pathway. *Biochemical*
696 *and Biophysical Research Communications*. **488** (1), 29–32 (2017).
697 44. Sun, T. et al. Radiosynthesis of 1-[^{18}F]fluoroethyl-L-tryptophan as a novel potential amino
698 acid PET tracer. *Applied Radiation and Isotopes*. **70** (4), 676–680 (2012).
699 45. Mock, B. H., Winkle, W., Vavrek, M. T. A color spot test for the detection of Kryptofix 2.2.2
700 in [18F]FDG preparations. *Nuclear Medicine and Biology*. **24** (2), 193–195 (1997).
701 46. Kim, D. W., Jeong, H. J., Lim, S. T., Sohn, M. H. Recent trends in the nucleophilic [^{18}F]-
702 radiolabeling method with no-carrier-added [^{18}F]fluoride. *Nuclear Medicine and Molecular*
703 *Imaging*. **44** (1), 25–32 (2010).

Figure 1

Click here to access/download;Figure;Figure 1.pdf



- Favorable half-life, no onsite cyclotron required
- Less radiolabeling precursor and reaction solvent
- Low loading of toxic K222
- 1% water used for [^{18}F]fluoride elution
- Injectable mobile phase for formulation

Figure 2

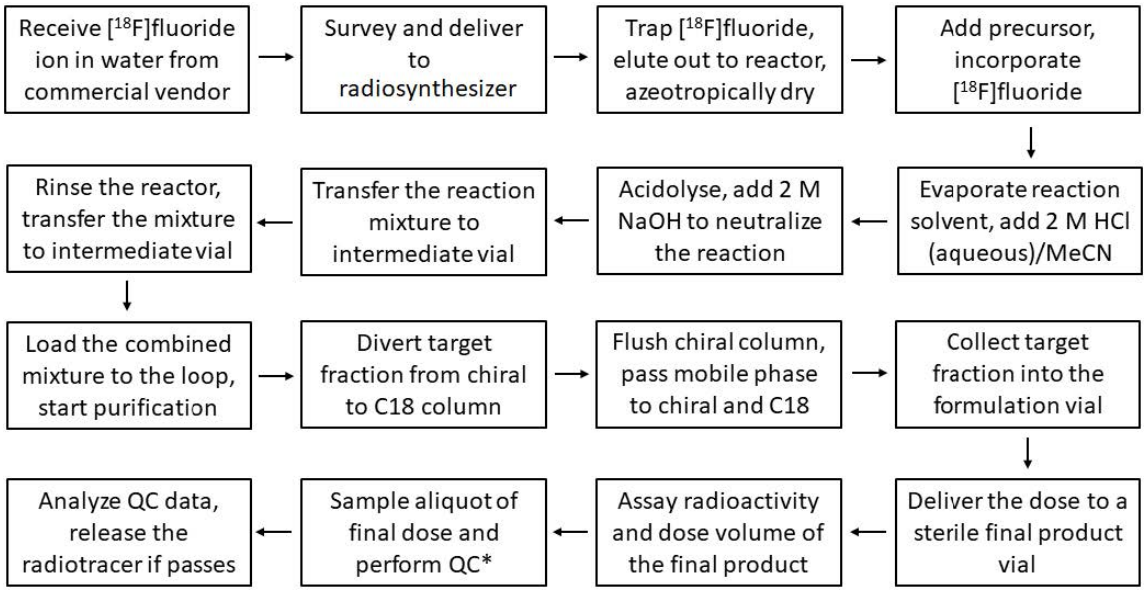
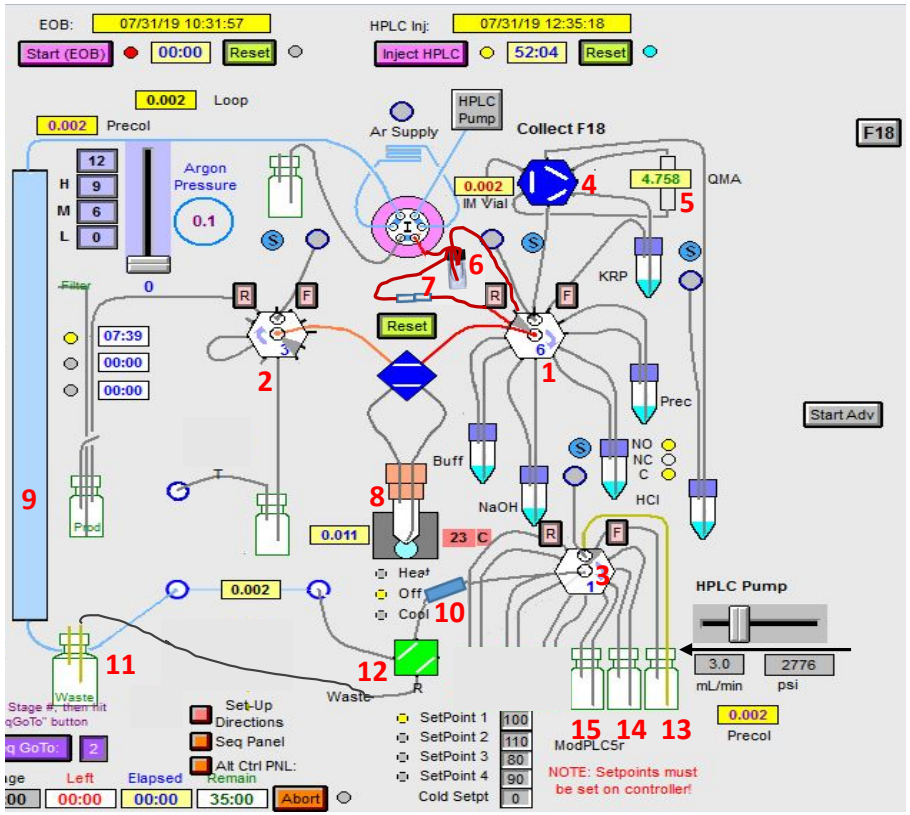


Figure 3. Production of L-[18F]FETrp.

Click here to access/download;Figure;Figure 3.pdf



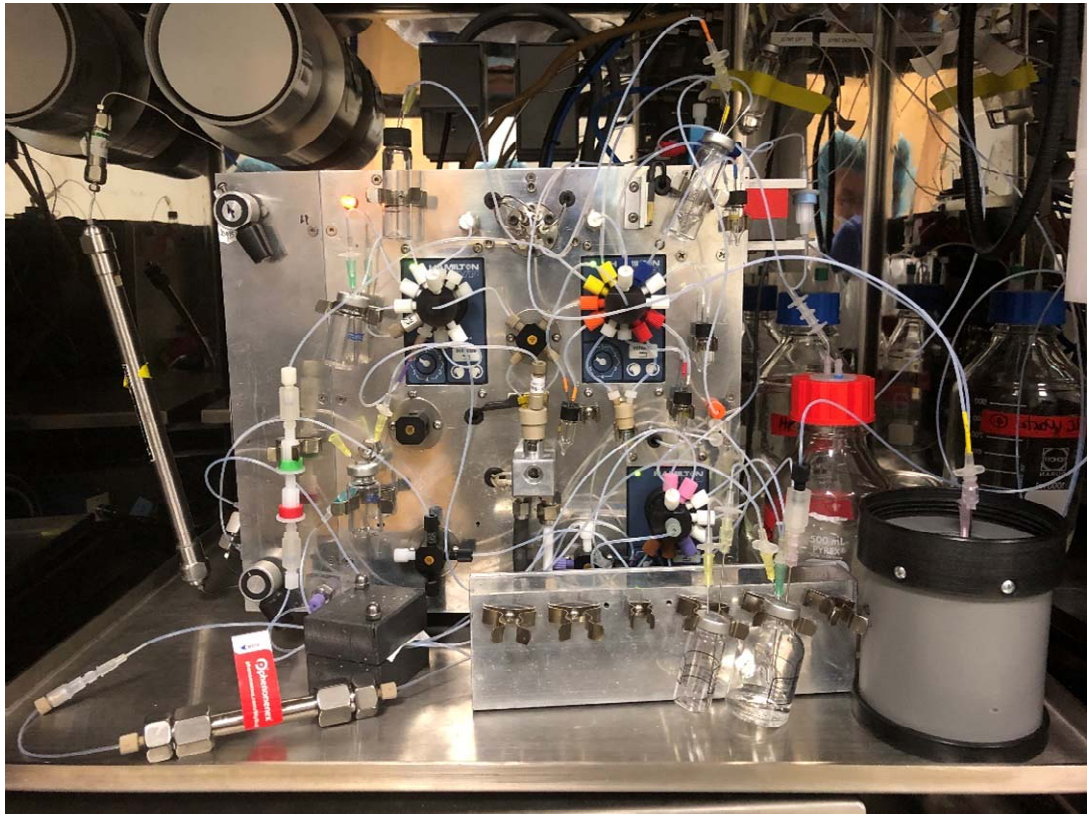


Figure 4. Typical semi-preparative chromatogram for purification of L-[18F]FETrp.

Click here to access/download;Figure;Figure 4.pdf

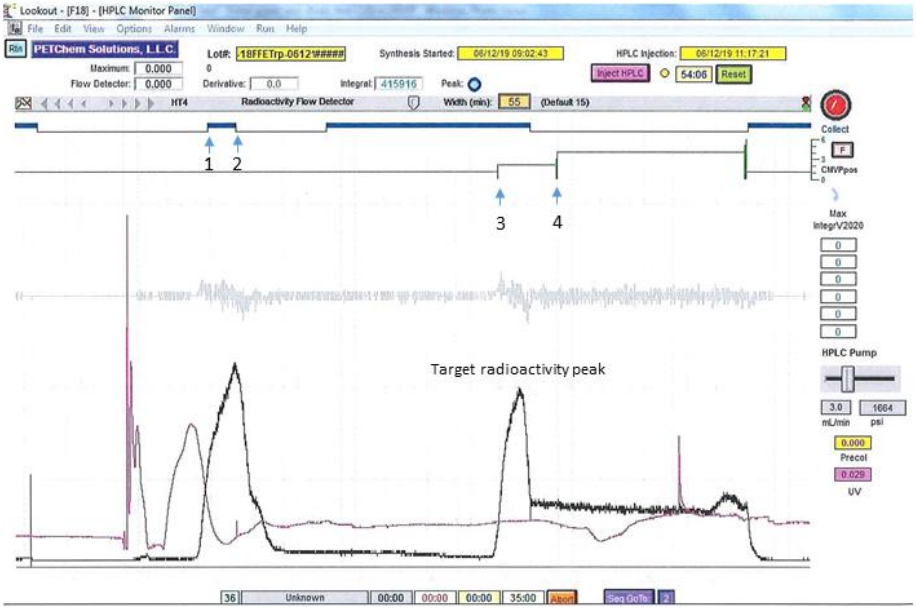
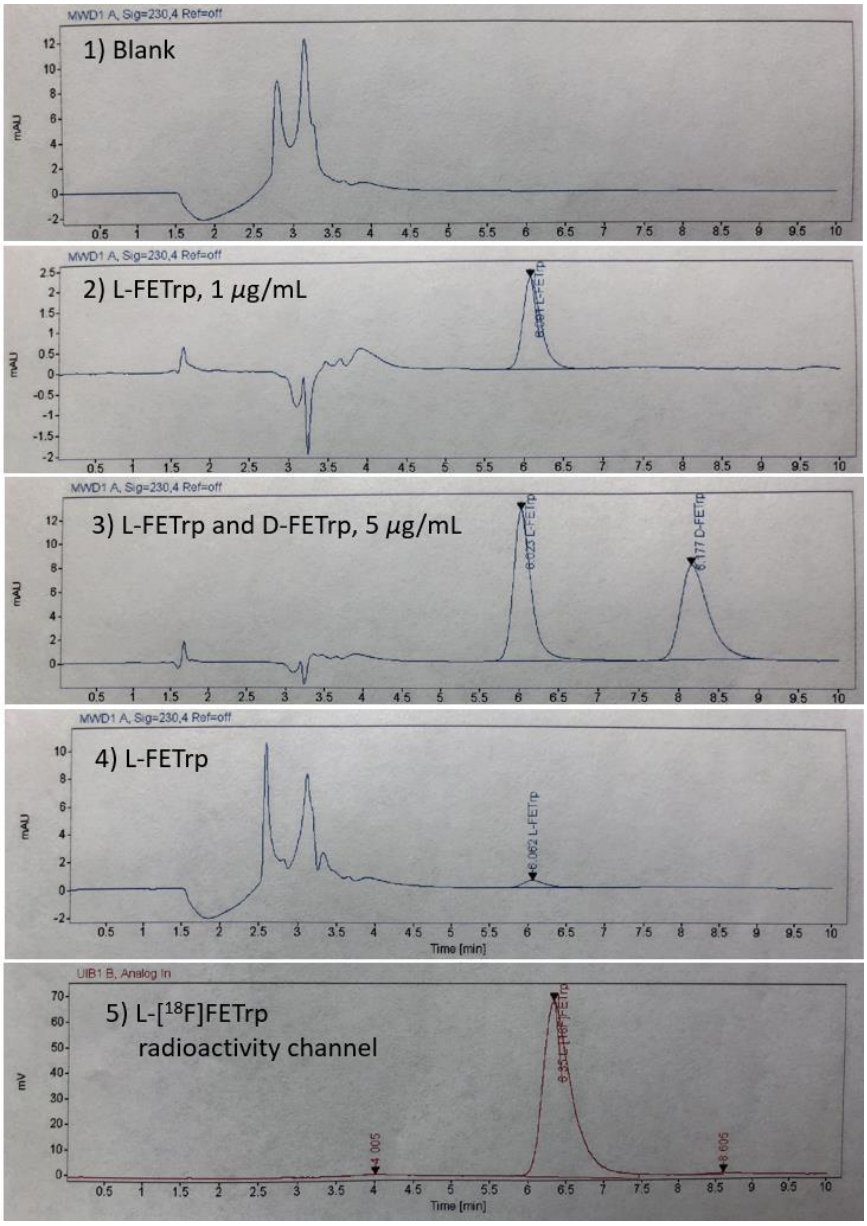


Figure 5. Typical analytical HPLC chromatogram for quality control of L-[18F]FETrp.



Tracer	Clinical Investigation	Major Indications	Pros	Cons
^{11}C-5-HTP	Yes	Imaging serotonin-producing neuroendocrine tumors, neuropsychiatric diseases	Sensitive in detecting small neuroendocrine tumors	Need an onsite cyclotron, short half-life, laborious procedures, multi-enzymatic radiosynthesis, sensitive to precursor concentration and solution pH, unspecific uptake in dopaminergic and noradrenergic areas
[^{11}C]AMT	Yes	Localization of epileptogenic tissue and brain tumors based on strong kynurenine pathway activations	cGMP production available, not incorporated into protein synthesis	Need an onsite cyclotron, short half-life, laborious procedures, complicated quantification under pathological conditions
L-[^{18}F]FETrp	No	Imaging kynurenine pathway including epileptic foci, brain tumors, and detecting epilepsy-associated neuroinflammatory abnormalities	Favorable half-life, available for satellite delivery, cGMP radiosynthesis, high stability towards defluorination and favorable radiation dosimetry	Uptake facilitated by both L-amino acid transporter and alanine-serine-cysteine transporter, no human investigations yet

Table 2. Stability test of L-[18F]FETrp in a typical batch at the highest dose concentration.

Hours After End of Synthesis of Assay	Radiochemical Purity by HPLC (%)	Chemical Purity (%)	Enantiomeric Excess (%)	pH Value
0	99	>95	98	5.5
1	99	>95	99	5.5
2	99	>95	99	5.5
4	99	>95	98	5.5
6	98	>95	98	5.5
8	97	>95	97	5.5

Click here to access/download

Table of Materials

Copy of JoVE_Materials-080921.xls

Response to the editorial comments

On behalf of the co-authors, I appreciate the time the editor took to provide insightful comments on our manuscript. We have uploaded a version of the manuscript with the following tracked changes or clarifications. Please let us know if any further changes are required.

1. Accepted the deletion of “respectively” in **1.3.2.** as the editor suggested.
2. Highlighted “15 ± 3 gigabecquerel (GBq) at the start of synthesis, see the Table of Materials” in **2.2.1.1.**
3. Accepted the replacement of PETCHEM synthesizer with radiosynthesizer in **Figure 2**.
4. We modified **Figure 3** according to the editor’s suggestions.