

One-pot Synthesis of High Molar Activity 6-^[18F]Fluoro-L-DOPA by Cu-Mediated Fluorination of a BPin Precursor

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1. General Considerations

Unless otherwise stated, reagents and solvents were commercially available and used without further purification: O-MOM-N-Boc-protected Bpin precursor (Part No. 1312) and authentic reference standards of 6F-I-DOPA (Part No. 1310), 6F-d,I-DOPA (Part No. 1311), and 6OH-d,I-DOPA (Part No. 1332) were purchased from ABX. 6H-I-DOPA (Part No. D9628), anhydrous pyridine (Part No. 270970), Tetrakispyridine copper(II) trifluoromethanesulfonate (Part No. 734527), ascorbic acid (Part No. 255564), hydrochloric acid (Part No. h1758), Tetrabutylammonium trifluoromethanesulfonate (Part No. 86888), and cesium carbonate (Part No. 441902) were purchased from Sigma Aldrich. Anhydrous N,N-dimethylformamide was purchased from Acros (Part No. 448381000). HPLC-grade Acetonitrile (Part No. A998-4), potassium acetate (Part No. P171-500), acetic acid (Part No. A38S-500), and sodium bicarbonate (Part No. S233-500) were purchased from Fisher Scientific. Ethanol (200 proof, USP) was purchased from Decon Laboratories, Inc. Sodium chloride 0.9%, USP and sterile water for injection, USP were sourced from Hospira. Other synthesis components were obtained as follows: Sterile vials were obtained from Hollister-Stier, Millex filters were from Millipore (Part No. SLFG025LS and SLGV013SL for GV and FG, respectively), and QMA-light cartridges were purchased from Waters. Luna NH2 5 micron 10x250mm and 4.6x150mm HPLC columns (Part No. 00g-4378-n0 and 00f-4378-e0), Luna NH2 guard cartridge discs (Part No. 00G-4454-N0PRP-214513), and Strata® 200mg SPE cartridges (Part No. 8B-5009-FBJ) were purchased from Phenomenex. Astec® CHIROBIOTIC® T Chiral HPLC column (Part No. 12024AST) was purchased from Sigma Aldrich. QMA cartridges were conditioned with ethanol, 0.5M NaHCO₃, and sterile water (10 mL of each, in that order) prior to use. Strata cartridges were conditioned with ethanol, sterile water, and acetonitrile (10 mL of each, in that order) prior to use.

Safety and hazards: All hazardous laboratory chemicals were used by trained personnel under the supervision of University of Michigan (UM) Environmental Health and Safety. Radioactivity was used by trained personnel under the approval of the UM Radiation Policy Committee (Protocol 12-029) and supervision of the UM Radiation Safety Service.

2. Radiosynthesis of [¹⁸F]6F-I-DOPA

The synthesis of [¹⁸F]6F-I-DOPA was fully-automated using a General Electric (GE) TRACERLab FX_{FN} synthesis module (Figure S1) loaded as follows: V1: 500 μL 15mg/mL TBAOTf + 0.2 mg/mL Cs₂CO₃ in water; V2: 1000 μL acetonitrile; V3: 4 μmol Bpin precursor, 20 μmol Cu²⁺, 500 μmol pyridine in 1 mL DMF; V4: 0.2 mL 0.25 M ascorbic acid + 0.6 mL 12.1 N HCl; V6: 3 mL acetonitrile; V7: 10 mL 0.9% saline, USP; V8: 2 mL ethanol, USP; Dilution flask: 100 mL acetonitrile ; F18 separation port: QMA cartridge ; C18 port: Strata cartridge.

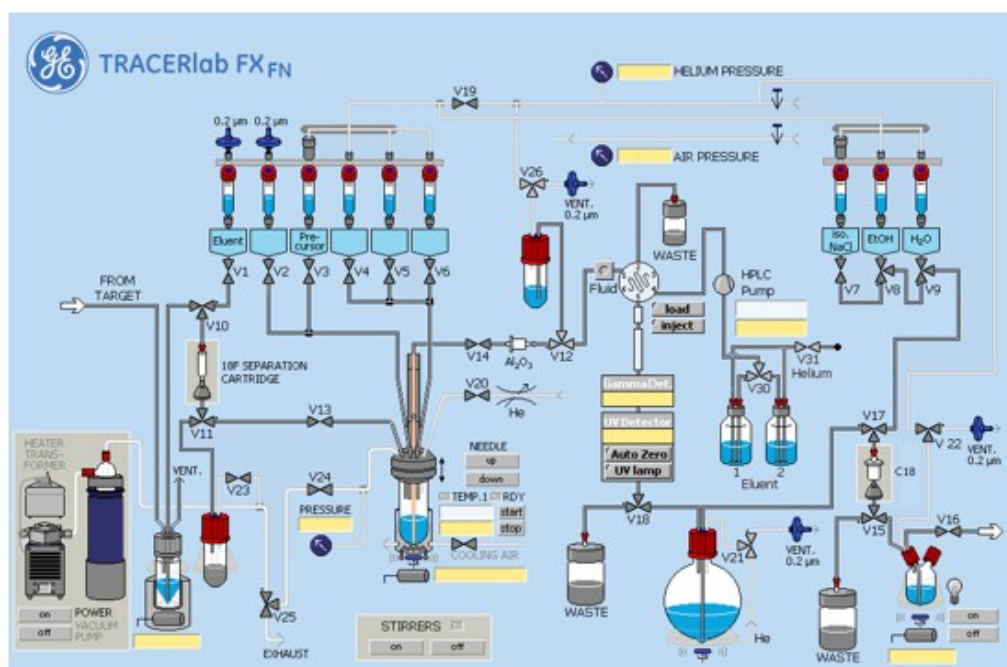


Figure S1. Synthesis of [¹⁸F]FDOPA on a GE TRACERLab FX_{FN}

[¹⁸F]Fluoride (~1800 mCi) was produced via the ¹⁸O(p,n)¹⁸F nuclear reaction by using a GE PETtrace cyclotron equipped with a high-yield fluorine-18 target. [¹⁸F]fluoride was delivered in a 1.5-mL bolus of [¹⁸O]H₂O to the synthesis module and trapped on a QMA-Light Sep-Pak to remove [¹⁸O]H₂O. [¹⁸F]Fluoride was then eluted into the reaction vessel with TBAOTf/Cs₂CO₃ eluent (V1). Acetonitrile (1 mL, V2) was added to the reaction vessel, and the [¹⁸F]fluoride was azeotropically

dried by heating the reaction vessel to 100 °C and drawing full vacuum for 6 min. After this time, the reaction vessel was subjected to both an argon stream and a simultaneous vacuum draw for 9 min at 100 °C. The reactor was cooled to 50 °C and the reactant solution (V3) was added to the dried [¹⁸F]fluoride using Ar push gas and stirred for five minutes at 50 °C to dissolve the dried [¹⁸F]fluoride. The temperature was then increased to 110 °C for twenty minutes. The reaction mixture was then cooled to 50 °C, deprotectant/antioxidant (V4) was added and the mixture was stirred at 110 °C for ten minutes. Subsequently, the reaction mixture was cooled to 50 °C, diluted with acetonitrile (V6, 3 mL), and purified by semi-preparative HPLC (column: Phenomenex Luna NH₂, 5 micron, 10x250 mm; mobile phase #1 (first 13 min): 90% acetonitrile 10mM KOAc pH: 7.5 ± 0.2; flow rate: 3 mL/min (first 3 min) and 5 mL/min (next 10 min); mobile phase #2 (after 13 min): 75% acetonitrile 10mM KOAc pH: 5.3 ± 0.2; flow rate: 5 mL/min; UV: 254 nm; see Figure S2 for a typical semi-preparative trace). The product peak (t_R ~22–23min) was collected into the dilution flask where it was concomitantly diluted with 100 mL of acetonitrile. The resulting solution was passed through a Strata® 200mg SPE cartridge, dried with Ar push gas for approximately three minutes, which was then washed with USP ethanol (V8, 2 mL) to remove any residual acetonitrile. [¹⁸F]6F-I-DOPA was eluted with 10 mL of 0.9% sodium chloride (USP for injection) and collected in the Tracerlab FX_{FN} product vial. The final drug product was dispensed into a septum sealed, sterile, pyrogen-free glass vial through a 0.22 µm sterile filter (Millex GV) and submitted for QC testing as outlined in the Quality Control testing (see Section 3 on Page S5).

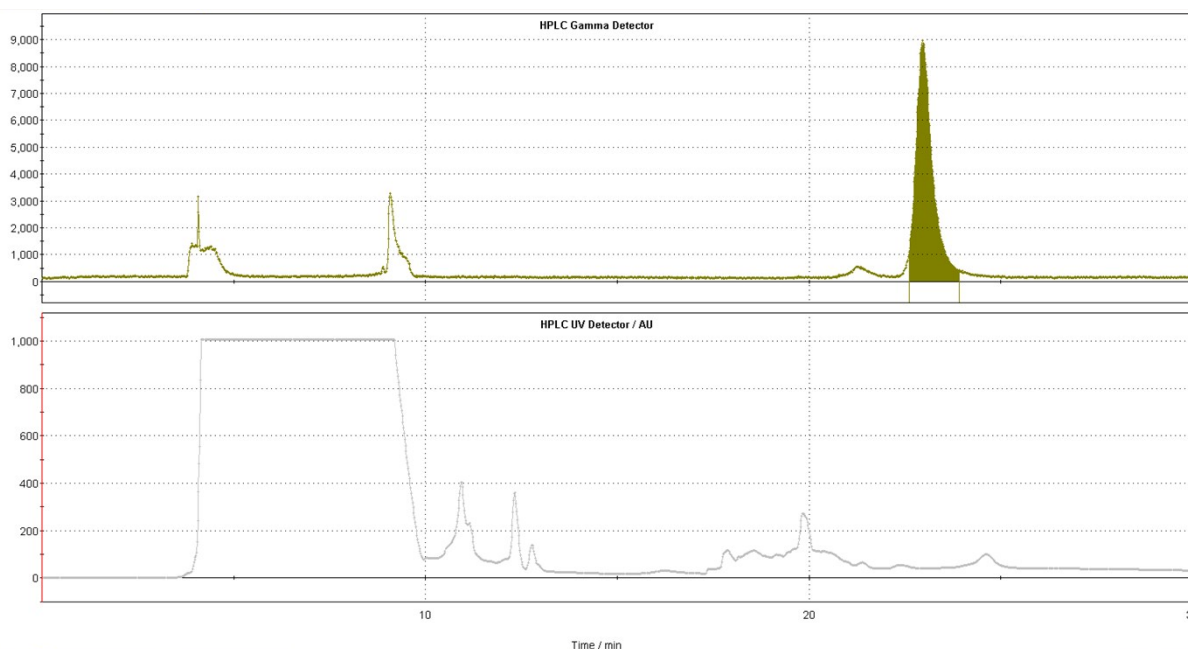


Figure S2. [¹⁸F]6F-I-DOPA Semipreparative trace (RAD top, 254nm UV bottom)

3. Quality Control testing of [¹⁸F]6F-I-DOPA

Quality control testing of [¹⁸F]6F-I-DOPA doses was conducted according to the guidelines outlined in the U.S. Pharmacopeia and as described below. Testing included visual inspection, pH, residual tetrabutylammonium, chemical purity and radiochemical purity (RCP), enantiopurity, molar activity, radionuclidic identity, residual solvent analysis (for acetonitrile and N,N-DMF), sterile filter integrity, bacterial endotoxin analysis, and sterility testing. Results for three process verification batches are reported in Table 1 in the main manuscript.

Visual inspection

Doses were visually examined and needed to be clear, colorless and free of particulate matter.

Dose pH

The pH of the [¹⁸F]6F-I-DOPA doses was analyzed by applying a small amount of the dose to pH-indicator strips and determined by visual comparison to the scale provided. Dose pH was required to be between 4.5 and 7.5.

Residual tetrabutylammonium (TBA⁺)

Residual TBA⁺ levels in [¹⁸F]6F-I-DOPA doses were analyzed using Dragendorff stain in order to conform to a European Pharmacopeia (EP) standard acceptable level of <0.26 mg/mL TBA⁺ (as of December 2018, no USP standards exist for TBA⁺).¹ Strips of glass-backed silica gel TLC plates were spotted with water (negative control), 0.26 mg/mL TBAOTf standard (positive control) and with the [¹⁸F]6F-I-DOPA dose. An air stream was used to dry the spots for one minute. The plate was dipped in Dragendorff reagent (prepared according to Bregoff *et al.*²) for 10 seconds, or until color developed on the spots. Plates were removed, air dried, and visually inspected. Orange spots indicated formation of TBA tetraiodobismuthanuide ([NBu₄⁺][BiI₄⁻]) precipitate. The limit of detection of the stain was determined to be 100 µg/mL using TBA standards prepared via serial dilution in normal saline, (10 mg/mL to 1µg/mL). No color alteration of the formulated [¹⁸F]6F-I-DOPA dose spot indicated < 260 µg/mL TBA present in the final dose, meeting European Pharmacopeia standards.¹

Chemical purity and radiochemical purity/identity

Chemical and radiochemical purities/identities were analyzed using a Shimadzu LC2010 HPLC equipped with a radioactivity detector and an ultraviolet (UV) detector (column: Luna NH₂ 5 micron

4.6x150 mm column; mobile phase: 70% MeCN 10 mM KOAc, pH 5.2; flow rate: 1.5 mL/min). A representative analytical HPLC trace is shown in Figure S3, and with an overlay of the UV trace of the reference standard in Figure S4. On this QC HPLC system, [^{18}F]6F-I-DOPA had a retention time of 4 – 5 min. Radiochemical purity for doses was confirmed to be >90%, and identity was confirmed by comparing the retention time of the radiolabelled product with that of the corresponding unlabelled reference standard.

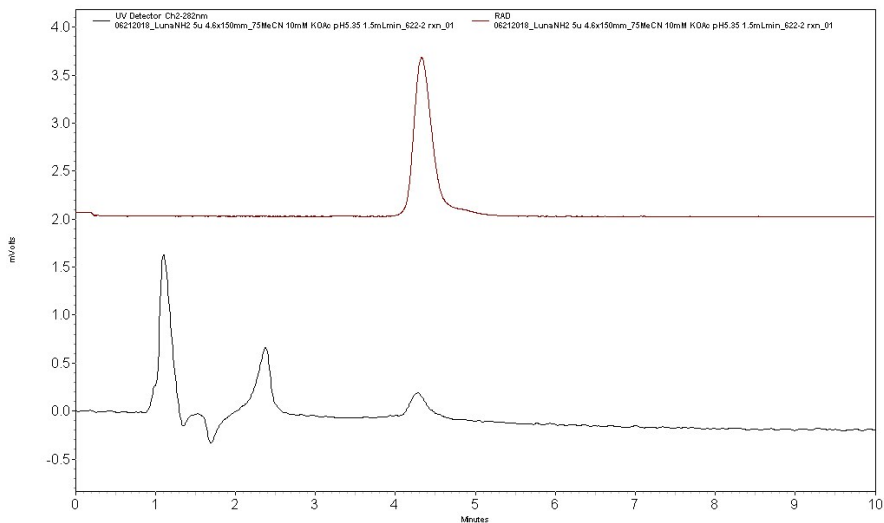


Figure S3. [^{18}F]6F-I-DOPA Analytical identity trace (RAD top, 282nm UV bottom) using a Luna NH_2 analytical column.

Enantiomeric Purity

Enantiomeric purity was determined by chiral HPLC using a Shimadzu LC2010 HPLC equipped with a radioactivity detector, an ultraviolet (UV) detector (column: Astec Chirobiotic T 5 micron 250 x 4.6 mm analytical column; mobile phase: 30% ethanol 10mM KOAc pH 5.13, flow rate: 1.5mL/min) and comparison to 6F-d,l-DOPA reference standard (Figure S4). Enantiomeric purity of doses was found to be >99%.

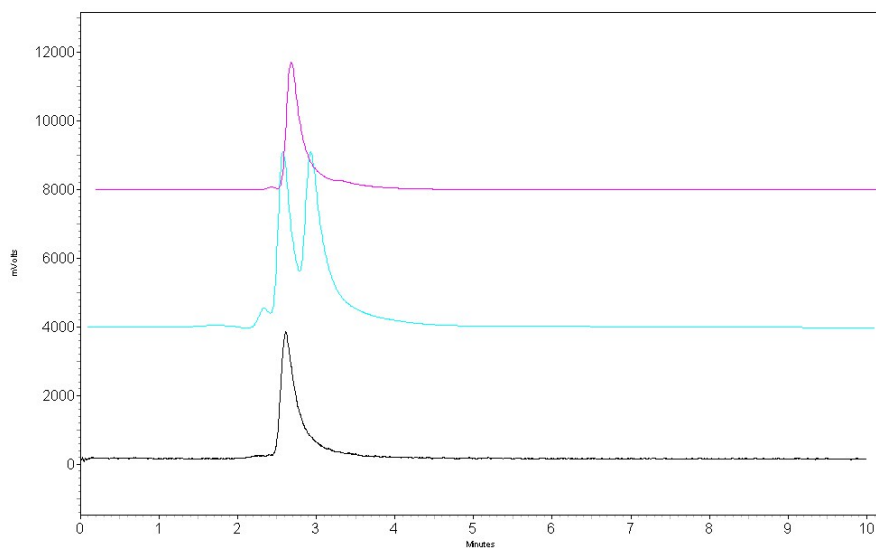


Figure S4. [^{18}F]6F-l-DOPA Analytical chiral trace of production dose (RAD, bottom, black) 6F-d,l-DOPA reference standard (282nm, teal, middle) and 6F-l-DOPA reference standard (282nm, pink, top) using a Chirobiotic T analytical column.

Residual Solvent Analysis

Levels of residual solvents in [^{18}F]6F-l-DOPA doses were analyzed using a Shimadzu GC-2010 with an AOC-20 autoinjector, split/splitless inlet, a flame ionization detector, and a Restek column (Stabilwax 30 m 0.25 mm, 0.25 m G16 stationary phase). Limits of residual solvents are based upon the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines (MeCN: ≤ 410 ppm; DMF: ≤ 880 ppm).³

Radionuclidic Identity

Radionuclidic identity was confirmed by determining the half-life of [^{18}F]6F-l-DOPA doses and comparing it to the known half-life of fluorine-18 (109.77 min). Activities were measured using a Capintec dose calibrator and half-life was calculated using Eq. (1). Calculated half-life must be 105–115 min.

$$T_{1/2} = -\ln 2 (\text{Time Difference} / (\ln(\text{ending activity}/\text{starting activity}))) \quad (1)$$

Sterile filter integrity (Bubble Point) test

Sterile filters from doses (with needle still attached) were connected to a nitrogen supply via a regulator. The needle is then submerged in water and the nitrogen pressure gradually increased. If the pressure was raised above the filter acceptance pressure (50 psi for 0.9% saline) without seeing a stream of bubbles, the filter was considered intact.

Bacterial endotoxins

Endotoxin content in [¹⁸F]6F-I-DOPA doses was analyzed by a Charles River Laboratories EndoSafe® Portable Testing System and according to the US Pharmacopeia. Doses must contain ≤175 Endotoxin Units (EU), or ≤17.5 EU/mL.

Sterility

Culture tubes of fluid thioglycolate media (FTM) and tryptic soy broth (TSB) were inoculated with samples of [¹⁸F]6F-I-DOPA and incubated (along with positive and negative controls) for 14 days. FTM was used to test for anaerobes, aerobes and microaerophiles while TSB was used to test for non-fastidious and fastidious microorganisms. Culture tubes were visually inspected on the 3rd, 7th and 14th days of the test period and compared to the positive and negative standards. Positive standards needed to show growth (turbidity) in the tubes, and [¹⁸F]6F-I-DOPA doses/negative controls had to show no culture growth after 14 days to be indicative of sterility.

4. HPLC Method Development for [¹⁸F]6F-I-DOPA

Prior to conducting any chemistry, we sought to develop a robust HPLC system that would enable the separation of [¹⁸F]6F-I-DOPA from reactants and potential by-products (6OH-I-DOPA and 6H-I-DOPA) and allow for visualization of the same during quality control. Prior reports utilized reverse-phase C18 HPLC columns for this task, however we found these to be unsatisfactory due to the close retention times of [¹⁸F]6F-I-DOPA and by-products. As an alternative, several hydrophilic interaction liquid chromatography (HILIC) columns were evaluated, including Phenomenex Polar-RP, Hydro-RP, and Luna NH₂ columns, as well as Quantofix ZIC- and BEH-HILIC columns. Of these, the best result was achieved using a Phenomenex Luna NH₂ column with a 5 micron resin bead diameter and an eluent with a high organic content: 70-75% acetonitrile incl. 10mM KOAc buffered with acetic acid to pH: 5.0-5.5 (near the hypothetical isoelectric point of 6F-I-DOPA). This system enabled adequate retention time differences of 6F-I-DOPA standard and 6-OH and 6-H-I-DOPA byproducts with both semipreparative and analytical columns (see Figures S5 and S6, respectively).

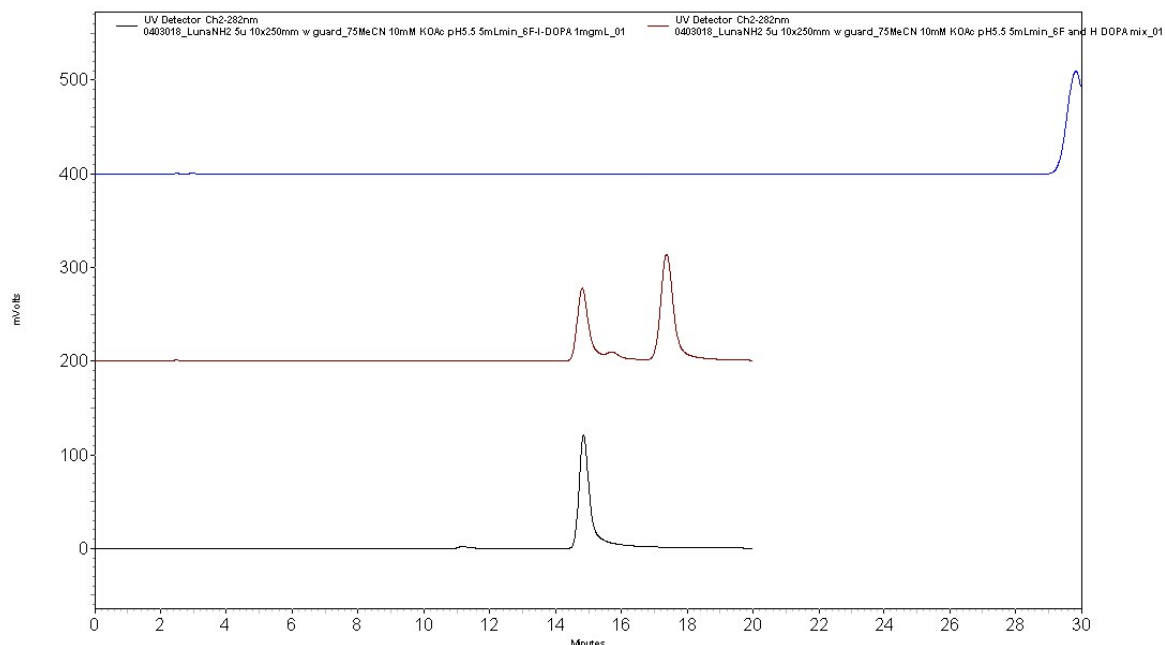


Figure S5: Retention time comparison of 6F-DOPA (bottom, black), 6H-DOPA + 6F-DOPA (middle, red) and 6OH-DOPA (top, blue) using a Luna NH₂ semipreparative column (282 nm, 70% MeCN 10 mM KOAc pH: 5.5 5 mL/min).

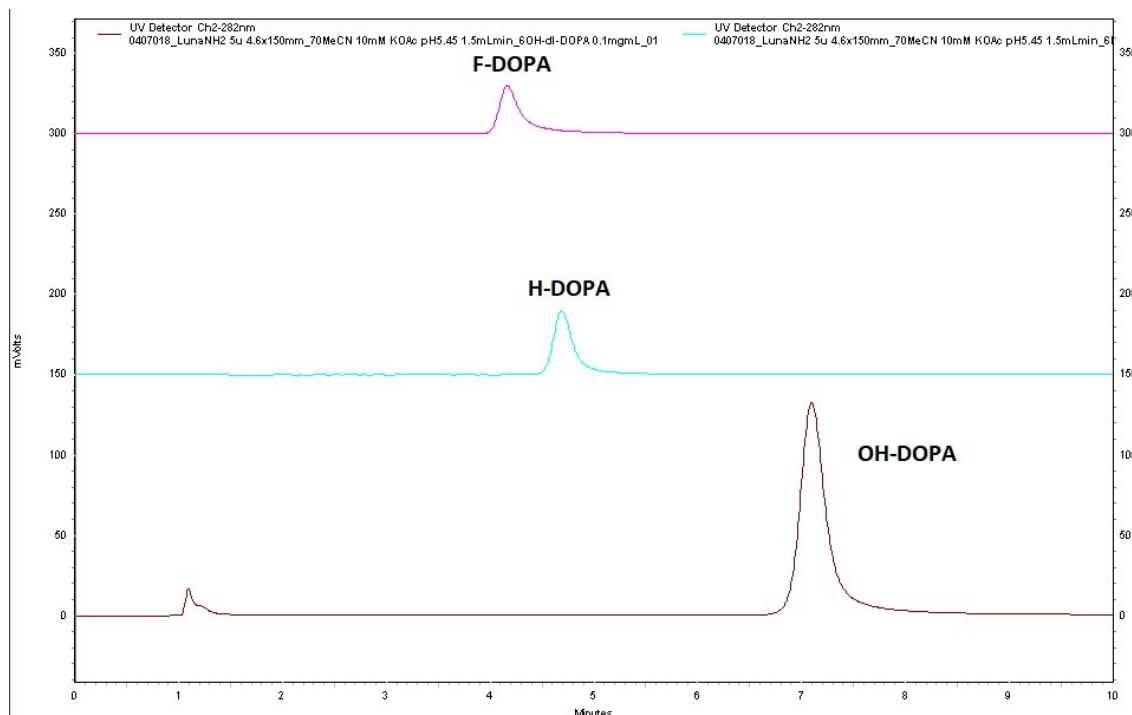


Figure S6: Retention time comparison of 6F-DOPA (top, pink), 6H-DOPA (middle, blue) and 6OH-DOPA (bottom, red) using a Luna NH₂ analytical column (282 nm, 70% MeCN 10 mM KOAc pH: 5.45 1.5 mL/min).

5. References

¹ The European Pharmacopoeia. European Directorate for the Quality of the Medicines (EDQM); Strasbourg, France: 2019, Version 10.0.

² H. M. Bregoff, E. Roberts and C. C. Delwiche, *J. Biol. Chem.*, 1953, **205**, 565–74.

³ Connelly, J. ICH Q3C Impurities: Guideline for Residual Solvents. In ICH Quality Guidelines by A. Teasdale, D. Elder and R. W. Nims (Eds) John Wiley & Sons, Inc. 2018, pp 199-232.