Supplemental Information:

Multi-GBq Production of the Radiotracer [¹⁸F]Fallypride in a Droplet Microreactor

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1 Materials

Anhydrous methanol (MeOH, 99.8%), ethanol (EtOH, 99.5%), anhydrous acetonitrile (MeCN, 99.8%), 2,3-dimethyl-2-butanol (thexyl alcohol, 98%), ammonium formate (NH₄HCO₂, 97%) and trimethylamine (TEA, 99%) sodium hydroxide (NaOH, 1N) were purchased from Sigma-Aldrich. Tetrabutylammounium bicarbonate (TBAHCO₃, 75mM), tosyl fallypride (fallypride precursor, >90%) and fallypride (reference standard for [¹⁸F]fallypride, >95%) were purchased from ABX Advanced Biochemical Compounds (Radeberg, Germany). Saline (0.9% w/v) was purchased from Hospira (Lake Forest, IL, USA). DI water was obtained from a Milli-Q water purification system (EMD Millipore Corporation, Berlin, Germany). No-carrier-added [¹⁸F]fluoride in [¹⁸O]H₂O was obtained from the UCLA Ahmanson Biomedical Cyclotron Facility.

2 Cartridge Fabrication

All resin types tested rely on guaternary ammonium functionality for trapping anionic species. Resin was packed into ETFE tubing with 1/16" outer diameter (OD) and different inner diameter (ID): 0.02" (1516L; IDEX Health and Sciences, Wallingford, CT, USA), 0.03" (1528L; IDEX) or 0.04" (1517L; IDEX). Loose resin was weighed on a balance (Excellence Plus, Mettler Toledo, Columbus, OH, USA) and placed within a 0.2 mL PCR tube (Fisherbrand, Fisher Scientific, Pittsburg, PA, USA). Tubing with desired inner diameter was cut to a length of 11 cm. A small polyethylene frit (1/8" thick, 20 micron pore size) was punched out of a larger disk (FT20751P, UCT, Inc., Bristol, PA USA) and was inserted into the tubing segment. Depending on tubing inner diameter (0.02", 0.03" or 0.04"), the frit was cut with a 0.5mm (504528, World Precision Instruments, Sarasota, FL, USA), 0.70mm (504529, World Precision Instruments), or 1.0mm (504646, World Precision Instruments) biopsy punch. Once the frit was inserted into the tubing, it was pushed down 4cm using the needle clearing rod from a spinal needle (Quincke Spinal Needle, BD Biosciences, San Jose, CA, USA) and secured within the tubing by pinching the tube to plastically deform the tube near the frit. The deformation was performed on the side of the frit closest to the opening in which the frit was loaded. Next, the same end of the tubing was connected to vacuum (-12 psi). For the Bio-Rad AG-MP1 and Sep-Pak QMA resins, slurries were made by adding 0.2mL of MeOH into the PCR tube with the measured resin. For Oasis MAX resin, a slurry was made with 0.2mL of DI water. Compared to DI water, the MeOH slurry was loaded into the cartridge tubing more smoothly with less fluidic resistance; however, for the Oasis

MAX resin, there was no difference between solvents. Next, the other end of the tubing was inserted into the bottom of the PCR tube to aspirate the slurry into the tubing. The frit served to trap the resin beads within the tubing. The PCR tube was then refilled with 0.2mL of the same solvent and aspiration repeated. Rinsing of the PCR tubing was performed a total of 2 times after the initial slurry loading. For cartridges packed with MeOH slurries, the cartridge was rinsed an additional time using 0.2mL DI water. After complete loading and rinsing, a second frit was punched, placed into the tubing, and is pushed right up to the resin bed. Finally, the tubing near this second frit was pinched to secure the frit and resin bed in place.

3 Cartridge Testing

Cartridge packing was assessed by flowing DI water through cartridges and measuring the flow rate. A sample reservoir (Falcon 15 mL conical tube, BD Biosciences, San Jose, CA, USA), flow sensor (SLI-2000, Sensirion Westlake Village, CA, USA), a cartridge to be tested, and a waste reservoir (Falcon 15 mL conical tube, BD Biosciences, San Jose, CA, USA) were connected in series with tubing (0.03"ID 1/16" OD ; 1528L, IDEX). 3mL of DI water was loaded into the sample reservoir. The sample reservoir was pressurized to 20 psi with a manual pressure regulator (ARX21-N01, SMC Corporation, Japan) connected to a nitrogen source. Flow rate of DI water through the cartridge was recorded (at 74 ms intervals) until the 3mL in the sample reservoir was depleted. An average flow rate and standard deviation was determined by averaging the last 500 samples taken.

4 Cartridge Preconditioning

We anticipated eluting the trapped [¹⁸F]fluoride with TBAHCO₃, the same phase transfer catalyst that we used in our previous work synthesizing [¹⁸F]fallypride in micro-droplets ¹, and performed preconditioning with 1M K₂CO₃.

In order to develop a preconditioning protocol, we fabricated cartridges using 0.03" ID tubing filled with 3mg of either Bio-Rad AG-MP1, Sep-Pak QMA resin, or Oasis MAX resin. Examining first the Bio-Rad AG-MP1 cartridges, we first flowed 0.5mL of preconditioning solution through the cartridge at 20 psi. Next, the cartridge was rinsed with DI water of different volumes. (The cartridge was left wetted after the rinse; no air drying was implemented.) Finally, we performed trapping of [¹⁸F]fluoride on the cartridge as described in the main paper to determine the impact of rinsing volume (**Table S1**). All solutions were flowed through the cartridge was quantitative. Lower amounts of rinse solution (4.0 or 6.0 mL) resulted in significantly worse trapping (i.e. 75% or 90%, respectively), perhaps due to small amounts of residual preconditioning solution that compete with [¹⁸F]fluoride for binding. We elected to use 10. mL of DI water for the rinse after preconditioning for the Bio-Rad AG-MP1 resin. We tried the same conditions with the other resins and found the trapping to be quantitative as well (**Table S1**).

Note that the preconditioning step can be performed during setup (prior to the introduction of the radionuclide) and thus the time needed does not adversely impact the overall radiochemical yield.

Table S1: Trapping efficiency of [¹⁸F]fluoride in cartridges with 3mg of varying resin as a function of volume of DI water rinse used during preconditioning Unless otherwise noted, data points represent n=1.

	Resin type						
	Bio-Rad AG-MP1				Sep-Pak QMA	Oasis MAX	
Water rinse volume (mL)	4.0	6.0	8.0	10.	10.	10.	

Trapping Efficiency (%)	75	90	99	99	99.8 ± 0.3 (n = 2)	99
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5 Droplet Merging Methods

5.1 Overview

Since the concentrated activity is eluted from the micro-cartridge as a series of 6.2 μ L plugs, the plugs are separated by air gaps. The initial plugs contain most of the concentrated activity, while lower amounts of activity are contained in later plugs. We were concerned that these gaps between plugs could impede efficient transfer to the downstream piezoelectric dispenser and/or adversely affect the dispenser performance. We therefore explored two methods to remove these air gaps; one involved the use of an intermediate vial and the other relied on the use of a microfluidic droplet merging chip.



Figure S1: Illustration of droplet merging methods used between the radionuclide concentrator and downstream dispenser. (A) Intermediate vial setup. (B) Droplet merging chip setup.

5.2 Intermediate Vial Approach

One approach to combine the sequential eluent plugs was to first collect them in a small intermediate vial, and then transfer the contents of the vial as a contiguous liquid plug to the reagent dispenser of the droplet-based synthesizer (**Figure S1A**).

The intermediate vial was comprised of a 250 µL glass insert (5181-1270, Agilent, Santa Clara, CA, USA) positioned within a septum-capped 1 mL v-vial (W986284NG, Wheaton, Millville, NJ, USA). The output of the concentrator was connected to the headspace of this vial via ETFE tubing (0.02" ID, 1/16" OD; 1516L; IDEX). An ETFE dip tube (0.01" ID, 1/16" OD; 1529L; IDEX) was inserted to the bottom of the intermediate vial and connected to the piezoelectric dispenser of the droplet synthesis platform. The end of the dip tube was cut at an angle to minimize dead volume at the bottom of the vial during fluid transfer. The dispenser was positioned ~5 mm above the radionuclide loading site of the reaction chip. To allow venting of the vial, we also inserted a needle (1" long, 25 gauge; 305125; BD Biosciences, San Jose, CA, USA) and connected this the common port of an electronic 3-way valve (LVM105R, SMC Corporation, Japan) via 1/8" OD

polyurethane tubing (TIUB01, SMC Corporation, Japan). One output of the valve was plugged and the other was vented to atmosphere.

During elution from the concentrator, this valve is vented to allow trapped air to escape from the intermediate vial. After completion of elution, this valve is closed (switched to the plugged output), and inert gas of the desired dispensing pressure is supplied by the concentrator to drive the vial contents toward the dispenser.

5.3 Microfluidic Chip Approach

To avoid potential losses due to splashing from the intermediate vial approach, we also explored the development of a microfluidic droplet merging chip to remove the air gaps (**Figure S1B**).

5.3.1 Chip design and operation

The chip (**Figure S2**) is comprised of three layers. The top layer ("fluid layer") was fabricated out of polyether ether ketone (PEEK) and has a fluid channel machined into it (1.6 mm wide, 0.63 mm deep, 30.3 mm long) along with two threaded inlet and outlet ports which can be interfaced to standard $\frac{1}{4}$ -28 fittings. The middle layer is a Teflon membrane with small (0.22µm) pores which serves as a gas-permeable membrane allowing air in between droplets to pass through to the other side while preventing the passage of aqueous liquids. The bottom layer ("vent layer") was machined out of acrylic and contains a channel matching the fluid layer (except depth was increased to 2.0 mm). The three layers were clamped together by eight M3 machine screws to form a liquid-tight seal.

Connections to and from the droplet merging chip are shown in **Figure S2D.** The output of the [¹⁸F]fluoride concentrator is connected to the fluid inlet port of the chip via ETFE tubing (0.01" ID, 1/16" OD; 1529L, IDEX). The fluid outlet port of the chip is connected via silicone tubing (1/32"ID, 1/16"OD; 05-14, Automate Scientific, Berkeley CA, USA) to a piezoelectric dispenser positioned in the synthesis platform. The dispenser was positioned ~ 5mm above the radionuclide loading site of the reaction chip (**Figure 3 of main paper**). Near the outlet of the droplet merging chip, the silicone tubing passed through a pinch valve (ASCO251866, ASCO Valves, Florham Park, NJ, USA). A pinch valve was chosen here due to the negligible dead volume compared to other valve types. In the vent layer, one vent port is plugged while the other vent port is connected to the common port of a 3-way valve (LVM105R, SMC Corporation, Japan) to enable switching between vented or plugged states.

During operation, the pinch valve is initially closed, and the vent layer is vented to atmosphere. The first liquid plug from the concentrator enters the chip (as trapped air escapes through the vent layer) but cannot travel to the outlet port. The elution process of the [¹⁸F]fluoride concentrator proceeds normally. As each plug is eluted, the eluent enters the fluid channel of the droplet merging chip and merges with the existing liquid as air in the air gap is pushed through the membrane layer. After all of the elution steps are complete and the elution plugs are merged, the pinch valve is opened and the vent outlet is plugged using the 3-way valve. Pressure is applied from the concentrator to push the merged eluent plug toward the reagent dispenser of the reactor chip.



Figure S2: Droplet merging chip. (A) Structure of assembled chip. (B) Photograph of assembled chip. (C) Bottom view of fluid layer, top view of membrane layer, and top view of vent layer, showing channel, inlet and outlet ports, and holes for securing screws. (D) Side view showing the three layers (with securing screws omitted for clarity) and connections of the chip to upstream radionuclide concentrator and downstream radiosynthesis platform.

5.3.2 Chip Performance

We characterized the residual activity (dead volume) of the droplet merging chip as a function of the number of elution plugs. A mock eluent solution was made containing [¹⁸F]fluoride at a known concentration and plugs of this mock eluent solution (each 6.2 μ L) were loaded into the droplet merging chip and merged using the procedure described above. The merged plug was collected in a small vial, and the recovered activity measured. Following each experiment, the chip was disassembled and cleaned to remove the possibility of carryover activity into subsequent experiments. The results are shown in **Figure S3**. When only one elution plug was passed through the chip, only 70 ± 6% (n = 3) of the activity was recovered. As the number of elution plugs was increased, the total amount of activity that was recovered increased. For 3 or 4 elution plugs, the activity recovery was high, i.e. 92.1 ± 5.1% (n=3) and 94.2 ± 1.9 (n=3), respectively.

It should be noted that these experiments were performed using plugs of uniform activity concentration. In real operation of the [¹⁸F]fluoride concentrator, later plugs would be more dilute and the loss of activity may in fact be lower than measured in these experiments.



Figure S3: Activity recovery from the droplet merging chip as a function of number of eluent plugs (each $6.2 \,\mu$ L) loaded into the chip. Each data point represents an average of 3 repeats with error bars representing standard deviation.

Ultimately, we found the performance of the droplet merging chip to be comparable to the intermediate vial, and thus opted to use the intermediate vial. The complicated operation (compared to the vial) and susceptibility for membrane breakthrough (if fluid pressure is too high or if fluid has too high an organic solvent content) reduce its practicality.

6 Evaluation of Synthesis Performance

Radioactivity was measured with a calibrated dose calibrator (CRC-25R, Capintec) at various times throughout the synthesis process-

Radioactivity recovery was calculated as the collected crude product divided by the starting radioactivity, corrected for decay. Fluorination efficiency of the collected crude product was determined via radio thin layer chromatography (radio-TLC). A 1 μ L droplet of crude product was spotted on a silica TLC plate (JT4449-2, J.T. Baker, Center Valley, PA, USA) with a micropipette. The TLC plate was developed in the mobile phase (60% MeCN in 25 mM NH₄HCO₂ with 1% TEA (v/v)) and then analyzed with a scanner (MiniGITA star, Raytest, Straubenhardt, Germany). In the resulting TLC chromatogram, two peaks were identified: unreacted [¹⁸F]fluoride (Rf=0.0) and [¹⁸F]fallypride (Rf=0.9). Fluorination efficiency was calculated as the area under the [¹⁸F]fallypride peak divided by the area under both peaks. The decay-corrected crude radiochemical yield (crude RCY) of [¹⁸F]fallypride was defined as the radioactivity recovery times the fluorination efficiency.

Cerenkov Luminescence Imaging (CLI) ² was utilized to visualize the distribution of radioactivity on the chip after drying the [¹⁸F]fluoride solution as previously described ¹. Briefly, to obtain an image, a glass microscope slide (1mm thick) was placed on top of the chip prior to placing it in the light-tight imaging chamber. An image was then collected (exposure time 300 s) and then image corrections and background subtraction were applied.

7 HPLC methods for Purification of [¹⁸F]fallypride

HPLC purification, and analysis of the purified product, were performed on an analytical scale Smartline HPLC system (Knauer, Berlin, Germany) equipped with a degasser (Model 5050), pump (Model 1000), a 200 μ L injection loop, a UV (254nm) detector (Eckert & Ziegler, Berlin, Germany) and a gamma-radiation detector and counter (B-FC- 4100 and BFC-1000; Bioscan, Inc., Poway, CA, USA). Separation was performed using a C18 column (Kinetex, 250 x 4.6 mm, 5 μ m, Phenomenex, Torrance, CA, USA). The mobile phase was 60% MeCN in 25 mM NH₄HCO₂ with 1% TEA (v/v) and flow rate was 1.5 mL/min. The retention time of fallypride was 4.5 min. Chromatograms were collected using a GinaStar analog-to-digital converter (raytest USA, Inc., Wilmington, NC, USA) and GinaStar software (raytest USA, Inc.) running on a PC. Based on the chromatograms, molar activity of [¹⁸F]fallypride was calculated as described previously ³.

8 Quality control testing

Quality control tests were performed on three batches of synthesized [¹⁸F]fallypride to ensure that the product is of sufficient quality for clinical use. These quality control tests include: total volume and activity concentration at end of synthesis, molar activity and carrier mass, appearance, sterile filter integrity, pH, radionuclide identity and purity, radiochemical identity and purity, chemical identity and purity, residual solvent analysis, residual content of tetrabutylammoniumbicarbonate (TBAHCO₃), bacterial endotoxins, and sterility. In addition, shelf-life of the formulated tracer was also examined. Methods for these quality control tests were based on our previous work ⁴, and are briefly described below.

8.1 Total volume and activity concentration at end of synthesis

Total volume of the collected product was estimated based on the graduations within the collection vial. Total activity was measured using a dose calibrator and the final activity concentration was calculated

8.2 Molar activity and carrier mass

Molar activity was determined as described above in section 8.

8.3 Appearance

After formulation, the product was examined behind leaded glass. The product was verified to be clear, colorless, and free of any particulate matter.

8.4 Sterile filter integrity

The integrity of the filter used to sterilize the formulated product was confirmed via a bubble point test. The output of the filter was connected to a bath of water as nitrogen pressure was applied to the input. The minimum pressure at which bubbling occurred was recorded, and should be > 50 psi.

8.5 pH

pH of the product was measured with pH paper (pH indicator strips #109542, pH 4.0 - 7.0 and pH indicator strips #109533, pH 5.0 - 10.0, EMD Millipore Corporation, Berlin, Germany). The pH of the final solution should be within 4.0 - 8.0.

8.6 Radionuclide identity and purity and gamma ray energy

The radionuclide identity was determined by measuring the half-life of the final sample of [¹⁸F]fallypride with a dose calibrator. Activity measurements were taken at various times (i.e. time 0, 10, 20, 30, 40 min) and half-life was calculated. A half-life of 104-115 min, corresponding to the half-life of fluorine-18 is required for the sample to pass.

Radionuclide purity of the synthesized [¹⁸F]fallypride was assessed with an ORTEC HPGe gamma ray spectroscopy system. The gamma ray energy measurement is carried out with a Nal(TI) based gamma spectrometer system (digiBASE) paired with ScintiVision-32 software (ALVtechnologies Philippines Incorporated, Pasig City, Philippines). Emission energy measured should be within 496-526 keV, the characteristic energy window for PET isotopes. For long-lived isotope content determination, the radiotracer is allowed to decay to background level activity before a sample is analyzed again. No more than 0.5% of decay-corrected activity should be attributed to impurities.

8.7 Radiochemical identity and purity

Radiochemical identity and purity tests of the formulated sample were performed using the analytical HPLC conditions described in the main paper. Prior to injection, the sample of purified tracer is spiked with the reference standard. To verify radiochemical identity, the radiochemical HPLC retention time of the product should match with the UV signal retention time of the reference standard. Radiochemical purity was determined from the HPLC chromatogram (gamma signal) as the area under the [¹⁸F]fallypride peak divided by the area under all peaks. HPLC conditions were described in the main paper. A radiochemical purity of > 95% is required to pass the radiochemically purity test.

8.8 Chemical Purity

5.8.1 UV impurities

The chemical purity of the formulated [¹⁸F]fallypride was determined with analytical HPLC using the conditions described in the main paper. The HPLC UV chromatogram was analyzed for impurity peaks. Any unidentified peak was quantified by making the assumption that the absorbance and molecular weight are the same as for fallypride. The sum of all impurities should be <1 μ g

8.8.1 Residual content of tetrabutylammoniumbicarbonate (TBAHCO₃)

Residual TBAHCO₃ in the purified sample was determined using a TLC spot test method reported by Kuntzsch *et al.* ⁵ A standard solution of TBAHCO₃ was created based on the injectable limit of TBAHCO₃ (i.e. 0.26 mg/mL for a 10 mL injection). The formulated [¹⁸F]fallypride was spotted (2 µL) onto a silica TLC plate (JT4449-2, J.T. Baker, Center Valley, PA, USA) alongside of a 2 µL spot of the TBAHCO₃ standard solution. The spots were allowed to dry and then each spot was covered with 10 µL of a developing solution (0.72M NH₄OH in 90% MeOH). The developing solution was allowed to dry and the TLC strip was inserted into a chamber containing iodine crystals for 1 min. The color intensity of the spot of the purified sample was compared to that of the standard solution to confirm the residual amount was below the injectable limit.

8.8.2 Residual solvent analysis

The concentration of residual solvents (i.e. methanol, acetonitrile, thexyl alcohol, and triethylamine) was determined using gas chromatography mass spectrometry (GCMS). Measurements were carried out using an Agilent 6890N GC, 5975 MSD, and 7683B Autosampler. The instrument was controlled by Enhanced Chemstation software version E.01.

The inlet was operated in split mode at 250 °C. Ultra-high purity He (Airgas West, Culver City, CA) was used as the carrier gas with the flowrate set to 1.2 mL / min. Separation was carried out on a 30 m x 250 µm x 0.25 µm DB-Wax column (Agilent J&W). The GC oven was initially held at 70°C, heated to 140 °C at 10 °C/min, and then heated to 260 °C at 30 °C/min. The MSD was operated in the SIM mode and used EI ionization.

Instrument response for known concentrations of pure analytes in water was measured to determine the analyte concentrations in the samples. More specifically, a two-point calibration

curve was generated for all four solvents at 10 PPM and 1000 PPM. The concentration of the residual analytes was then interpolated from this calibration curve. The maximum allowed concentrations for all the organic solvents used in the synthesis of [¹⁸F]fallypride are provided in **Table S5**.

8.9 Bacterial Endotoxins

The presence of bacterial endotoxins was tested using an Endosafe-PTS test kit (Charles River Laboratories, Wilmington, MA, USA). Bacterial endotoxin levels should be <175 EU/total batch.

8.10 Sterility

The sterility of the synthesized [¹⁸F]fallypride was determined through inoculation of a sample of the final product with both fluid thioglycolate medium (FTM) and tryptic soy broth (TSB). The inoculated FTM and TSB media were incubated at 32.5 °C and 22.5 °C, respectively, for 14 days. No growth in the cultures verifies the sterility of the final product.

8.11 Product shelf-life testing

In order to determine product stability on the shelf, the pH, appearance, and radiochemical purity of the final product were assessed after 5 hours. A passing result in all three tests confirms product stability with a 5 hour shelf-life.

9 Simulating higher activity levels

When performing trap and elute experiments, we saw some limitations with the 3 mg cartridge at higher activity levels, and therefore performed experimentation to explore what factors may be influencing trapping capacity in our cartridges.

9.1 KF Spiking

To simulate high starting activities (i.e., 37 GBq), we tried performing trapping experiments with low amounts of [¹⁸F]fluoride spiked into a potassium fluoride (KF) solution. Estimating the molar activity of [¹⁸F]fluoride from our cyclotron to be 740 GBq/µmol (based on prior measurments), the total amount of fluoride ion (both F-18 and F-19 forms) expected for 37 GBq [1 Ci] should be 0.05 µmol. Mock solutions were prepared using different concentrations of KF (1.0 mL volume) chosen from 50 – 5000 µM, which were expected to correspond to simulated activity ranges of 37 GBq to 3.7 TBq. These solutions were spiked with 50 µL of [¹⁸F]fluoride (15 - 37MBq [0.4 - 1mCi]), an amount of activity sufficient for accurate activity measurements, but contributing negligible fluorine ion compared to the KF content. The measured trapping efficiency for each of these solutions is shown in **Figure S4**.



Figure S4: Trapping efficiency of a solution containing 1.0 mL KF of various concentrations spiked with a small amount of [¹⁸F]fluoride. All trapping experiments were performed with micro-cartridges packed with 3 mg of Sep-Pak QMA resin. Each condition was tried once (n=1). Dotted line represents a logarithmic fit and has an $R^2 = 0.9922$.

Trapping efficiency of the mock 37 GBq [1 Ci] sample (i.e. 50 μ M KF) was 100% suggesting the cartridge should be able to trap 37 GBq [1 Ci] of radioactivity, but the trapping was observed to fall off rapidly as the amount of KF increased. For example, at a mock activity level of 740 GBq [20 Ci] (i.e., 1 mM KF), we can interpolate the measurements and estimate a trapping efficiency of ~40%.

9.2 Decayed, bombarded [¹⁸O]H₂O Spiking

We performed additional experiments using decayed, bombarded [¹⁸O]H₂O to evaluate whether the KF spiking measurements would be predictive of actual performance. We took 1.0 mL of decayed bombarded [¹⁸O]H₂O (taken from a bombardment when the activity at end of bombardment was ~37Gbq [1.0 Ci]). Though the [¹⁸F]fluoride had decayed, the total fluoride content was not expected to have significantly changed since the amount of [¹⁹F]fluoride right after bombardment far exceeds the amount of [¹⁸F]fluoride produced. We spiked in a small amount of activity (~19 MBq [0.5 mCi]) and performed a trapping experiment. Surprising, the trapping efficiency was found to be only 42%.

While we believe this experiment to be predictive of the results using 37 GBq of freshly produced [¹⁸F]fluoride, this result suggested that KF solution is not a good substitute for performing mock experiments. Furthermore, it suggests that there may be a high concentration of a competing species (e.g. anion, metals) present in bombarded [¹⁸O]H₂O that may also be interacting with the cartridge and limiting the capacity available for trapping fluoride.

Further study is needed to determine the impurities and how they may be hindering trapping. As mentioned earlier, other groups have reported efficient trapping of up to 110 GBq (3 Ci) using cartridges with only 2 mg of resin. (Our cartridges used 3 mg of resin.)

In the next section, we explore whether larger resin mass can improve the trapping capacity, and in the subsequent section, we explore the use of SCX cartridges to filter the bombarded [^{18}O]H₂O to try to remove interfering species.

10 Trap and Elute with Larger Resin Mass

To attempt to increase trapping capacity, we explored cartridges packed with higher resin masses, i.e. 5mg of either Sep-Pak QMA or Oasis MAX resin. Due to limited supply of high levels of [¹⁸F]fluoride or corresponding amounts of bombarded [¹⁸O]H₂O, we performed initial experiments with low amounts of activity spiked into a 1 mM KF solution (as described above). This KF concentration (corresponding to 740 GBq [20 Ci]) was chosen because the trapping efficiency in spiking experiments was ~40%, which matched the trapping efficiency in spiking experiments using 1.0 mL of decayed [¹⁸O]H₂O from 37 GBq [1 Ci] bombardment. We are aware that the low trapping efficiency from the bombarded [¹⁸O]H₂O spiking experiments may not entirely be due anion contamination, but, nonetheless, this experiment could still give us some insight on the trapping capacity of cartridges with larger resin amounts.

Detailed trapping and elution behavior can be seen in **Supplemental Material Table S2**. Compared to 42% trapping for the 3mg Sep-Pak QMA cartridge reported above, trapping efficiency was 71± 1% (n = 2) for 5 mg Sep-Pak QMA cartridges and $68\pm 5\%$ (n = 2) for 5 mg Oasis MAX cartridges. For 7 mg of Oasis MAX resin (packed in larger tubing as described in **Section 1**), trapping efficiency increased to 84% (n = 1), indicating that more resin mass can indeed improve the trapping.

One drawback of the larger resin mass is decreased elution efficiency. For the 5 mg cartridges, even after four elution steps, the elution efficiencies were $78\pm 1\%$ (n = 2) and $78\pm 1\%$ (n = 2) for Sep-Pak QMA and Oasis MAX resin, respectively. For the 7 mg Oasis MAX cartridge, elution efficiency was only 47% (n=1) after four elution steps. Comparing the overall recovery efficiency (i.e. trapping efficiency x elution efficiency (4 elutions)), the 5 mg Sep-Pak QMA cartridges performed the best (55.5 ± 0.4%, n = 2) compared to 5mg Oasis MAX (53 ± 4%, n = 2), and 7mg Oasis MAX (39%, n = 1).

	Resin type			
	Sep-Pak QMA	Oasis MAX	Oasis MAX	
Cartridge Mass (mg)	5	5	7	
Tubing ID (inch)	0.03	0.03	0.04	
Number of repeats (n)	2	2	1	
Trapping efficiency (%)	71 ± 1	68 ± 5	84	
Elution 1+2 Efficiency (%)	30 ± 2	27± 1	11	
Elution 3+4 Efficiency (%)	49 ± 1	50.3 ± 0.3	36	
Elution 5+6 Efficiency (%)	19.3 ± 0.1	21 ± 3	39	
Elution efficiency (4 elutions) (%)	78 ± 1	78± 1	47	
Recovery Efficiency (4 elutions) (%)	55.5 ± 0.4	53 ± 4	39	
Elution efficiency (6 elutions) (%)	98 ± 1	98± 3	86	

Table S2: Trapping and elution performance of a [18 F]fluoride solution spiked with 1mM KF using cartridges with increased resin mass. Values represent average ± standard deviation, calculated from the indicated number of repeats (n).

11 SCX filtration of [18F]fluoride

11.1 [¹⁸F]fluoride Filtering Testing

We explored whether passing the [¹⁸F]fluoride/[¹⁸O]H₂O solution through an SCX cartridge helped to improve the trapping efficiency of the downstream concentrator module. We hypothesized that SCX cartridges may filter out some competing contaminants and/or particles that may be interfering with the [¹⁸F]fluoride trapping efficiency.

Two different SCX cartridges were explored: Oasis MCX Plus short cartridges (225mg, 186003516, Waters, Milford, MA, USA) and Maxi-Clean cartridges (600mg, 21902, Alltech Associates Inc. Deerfield, IL, USA). Before use, SCX cartridges were first preconditioned via manufacturer recommendations. Alltech Maxi-clean cartridges were preconditioned with 10mL of DI water while Oasis MCX cartridges were conditioned with 5mL of MeOH followed by 5mL of DI water.

1.0 mL of decayed bombarded [¹⁸O]H₂O (1mL; original activity of ~37 GBq [~1 Ci]), was manually passed through a SCX cartridge via syringe, and then spiked with 50 µL of [¹⁸F]fluoride (15 -37MBg [0.4 - 1mCi]). The spiked solution was then used as the source solution for the radionuclide concentrator and the trapping efficiency was measured. For this experiment, we used preconditioned 3 mg or 5 mg micro-cartridges packed with Sep-Pak QMA resin. The results are listed in Supplemental Material Table S3. For the 3mg Sep-Pak QMA cartridges, SCX filtration (will Alltech Maxi-Clean cartridge) resulted in a dramatic increase in trapping efficiency from 42% (n = 1) to 78 ± 4% (n = 2). The trapping efficiency was even higher $(92 \pm 1\%, n=2)$ when the Oasis MCX cartridge was used for the SCX filtration step. For the 5 mg cartridges, this effect was less significant. Trapping efficiency increased from $81 \pm 4\%$ (n = 3) to $96 \pm 2\%$ (n = 4) after SCX filtration using the Alltech Maxi-Clean cartridge. Oasis MCX cartridges in conjunction with the 5mg Sep-Pak QMA cartridges were not tested. Interestingly, higher trapping efficiency could be obtained with 3 mg cartridges after SCX filtration, compared to trapping efficiency of 5 mg cartridges if SCX filtration was not performed. We elected to use the 3 mg Sep-Pak QMA cartridges in conjunction with SCX filtration (Oasis MCX cartridge) for the majority of experiments in the paper.

Despite the improved trapping of the spiked solutions using SCX cartridge filtration, trapping was lower than expected when using higher starting amounts of [¹⁸F]fluoride (i.e., > 10 GBq), suggesting that the spiked solutions are not a perfect surrogate for higher activity [¹⁸F]fluoride solutions. Future studies are needed to determine if higher resin masses, in conjunction with the SCX module, could consistently achieve high trapping efficiencies, as well as high elution efficiency with low eluent volume.

Table S3: Trapping performance of [¹⁸F]fluoride spiked into a solution of decayed [¹⁸O]H₂O that was filtered through an SCX cartridge or not filtered (indicated as "None" for SCX cartridge type). The micro-SAX cartridges were all packed with Sep-Pak QMA resin.

SCX cartridge type	None	Alltech Maxi-Clean	Oasis MCX	None	Alltech Maxi-Clean
Micro-SAX cartridge resin mass (mg)	3	3	3	5	5
Trapping Efficiency (%)	42 (n = 1)	78 ± 4 (n=2)	92 ± 1 (n=2)	81 ± 4 (n=3)	96 ± 2 (n=4)

11.2 Setup and Operation of SCX Filtration Module

The core of SCX filtration module (Figure S5) is a 7-port, 6-position rotary stream selection valve ("filtration selection valve", Titan HT 715-005, IDEX Health and Science). Connected to three inputs of this valve are three septum-capped glass v-vials (3mL, W986277NG, Wheaton, Millville, NJ, USA) for supplying [¹⁸F]fluoride and two rinses of DI water (each 0.5 mL). The remaining three inputs of the stream selection valve are not used. The vials are each connected to the valve via an ETFE dip tube (0.02" ID, 1/16" OD ETFE tubing; 1516L; IDEX), and are also connected to a regulated inert gas source or vented to atmosphere via 3-way valves (V1-V3) (S070B-5DG, SMC). Inert gas pressure was provided from an electronic pressure regulator (ITV0010-2BL, SMC Corporation, Japan) connected to a nitrogen source. The output of the stream selection valve is connected to an SCX cartridge and then to a collection vial (3mL v-vial; Wheaton) via 0.02" ID, 1/16" OD ETFE tubing (1516L, IDEX). Two strong cation exchange cartridges were explored; Oasis MCX plus short cartridges (225mg, 186003516, Waters), and Maxi-Clean cartridges (600mg, 21902, Alltech). The collection vial is also connected via a 3-way valve (LVM105R, SMC Corporation, Japan) to atmosphere or the regulated inert gas supply. Finally a dip tube from the collection vial is connected to the fluoride input port of the [18F]fluoride concentrator via 0.02" ID, 1/16" OD ETFE tubing (1516L, IDEX).

The use of the SCX filtration module required a slight modification of the [¹⁸F]fluoride trapping procedure. Initially the reagent selection valve of the SCX filtration module is set to the [18F]fluoride vial, and V4 is set to vent the collection vial of the module. V1 is connected to pressure (20 psi) pushing [¹⁸F]fluoride through the SCX cartridge into the SCX module collection vial. Immediately after, the reagent select valve is switched to connect to the first water rinse vial and V2 is connected to pressure (20 psi) to deliver 0.5 mL of DI water through the SCX cartridge to recover residual [¹⁸F]fluoride. The radionuclide concentrator is then configured in "trapping" mode, the reagent selection valve of the SCX filtration module is changed to an unused (plugged) position (to prevent pressure leakage from the SCX module collection vial), and V4 is switched to connect to pressure (20 psi) to drive the mixture of filtered [¹⁸F]fluoride and DI water rinse to the concentrator. When complete, V4 of the SCX filtration module is switched to vent the collection vial, the reagent select valve is connected to the second water rinse vial, and V2 is connected to pressure (20 psi) to flush the 0.5 mL of DI water through the SCX cartridge and into the collection vial. Finally, the reagent selection vial of the SCX filtration module is switched to an unused (plugged) position, and V4 is connected to pressure (20 psi) to drive the water to the radionuclide concentrator. Operation of the concentrator module then proceeds as described earlier.



Figure S5: Schematic of the SCX filtration module.

12 Optimization of Transfer Method

To optimize the transfer method from the radionuclide concentrator to the radiosynthesis chip, the components were disassembled after transfer, and activity left in the dispenser, activity loaded on the chip, activity left in the intermediate vial and activity left in the tubing and frit were measured to figure out the activity loss.

Table S4: Activity loss in various locations within the integrated system during [¹⁸F]fluoride transfer and dispensing relative to activity trapped during concentration.

	Distribution of radioactivity after elution			
	Without the 2nd DI water rinse	With the 2nd DI water rinse		
Activity left in the dispenser (%)	25.6	3.1		
Activity loaded on the chip (%)	48.2	95.9		
Activity left in the intermediate vial (%)	20.1	0.5		
Activity left in the tubing and frit (%)	1.5	0.5		



13 HPLC purification chromatograms

Figure S6. Examples of HPLC purification (crude) chromatograms of syntheses starting with activities of (A) 0.19 GBq [5 mCi], (B) 8.5 GBq [230 mCi], (C) 21 GBq [568 mCi], and (D) 41 GBq [1.1 Ci].



Figure S7. Examples of HPLC chromatograms of (A) formulated [¹⁸F]fallypride, (B) formulated [¹⁸F]fallypride after a 5 hour shelf-life test, (C) formulated [¹⁸F]fallypride coinjected with fallypride reference standard. The excipient is part of the formulation buffer, confirmed with HPLC.

14 Quality Control Testing Results

Table S5: Tabulated quality control tests highlighting testing criteria for each test, results for three different batches of synthesized [¹⁸f]fallypride, and overall QC result from all three trials.

Test	Testing Criteria	Trial 1	Trial 2	Trial 3	Result
Appearance	Clear, colorless, particle free	Pass	Pass	Pass	Pass
Radioactivity concentration	7.4-74 MBq/mL [0.2-2 mCi/mL]	52 MBq/mL [1.4 mCi/mL]	7.4 MBq/mL [0.2 mCi/mL]	37 MBq/mL [1.0 mCi/mL]	Pass
Molar Activity	> 37 GBq/umol [1 Ci/ umol]	260 GBq/ umol [7 Ci/ umol]	140 GBq/ umol [3.8 Ci/ umol]	240 GBq/ umol [6.5 Ci/ umol]	Pass
Chemical purity					
a) TBAHCO ₃	< 2.6mg/injection	< 2.6mg /injection	< 2.6mg /injection	< 2.6mg /injection	Pass
	TEA < 5000 PPM	< 10 PPM	< 10 PPM	906 PPM	Pass
h) Residual	MeCN < 410 PPM	< 10 PPM	< 10 PPM	< 10 PPM	Pass
solvents	MeOH < 3000 PPM	< 10 PPM	< 10 PPM	< 10 PPM	Pass
	Thexyl alcohol < 5000 PPM	< 10 PPM	< 10 PPM	< 10 PPM	Pass
Radiochemical identity	Retention time ratio of radio peak vs cold standard (0.9-1.1)	1.0	1.0	1.0	Pass
Radiochemical purity	> 95%	> 97%	> 99%	> 99%	Pass
Radionuclide identity	104-115 min	108	109	109	Pass
Gamma ray emission energy	496-526 keV photons	512 keV	516 keV	512keV	Pass
Radionuclide purity	No less than 99.5%	100%	100%	100%	Pass
рН	4.0-8.0	6	6	4.5	Pass
Filter integrity	> 50 PSI	> 50 PSI	> 50 PSI	> 50 PSI	Pass
Bacterial endotoxin	< 175 EU/total batch	< 20 EU	< 20 EU	< 20 EU	Pass
Sterility Stability on shelf	Sterile	Yes	Yes	Yes	Pass
a) Appearance	Pass after 240 minutes	Pass	Pass	Pass	Pass
b) pH	Pass after 240 minutes	Pass	Pass	Pass	Pass
c) Radiochemical purity	Pass after 240 minutes	Pass	Pass	Pass	Pass

15 References

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