Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Microfluidic continuous-flow radiosynthesis of [^{18}F]FPEB suitable for human PET imaging

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Supporting information

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Automated synthesis of [^{18}F]FPEB by GE TracerLab FX$_{FN}$ method

A GE PETtrace 16/8.5 MeV cyclotron was used for [^{18}F]fluoride radionuclide production. A GE high yield niobium target containing > 97% enriched O-18 water (Isotec, Taiyo Nippon Sanso or Rotem) was bombarded with protons at integrated currents up to 65 µA to produce [^{18}F]fluoride. Following completion of bombardment, the [^{18}F]fluoride was transferred to the GE TRACERlab™ FX$_{FN}$ radiosynthesis module via helium gas overpressure.

A schematic diagram of the GE medical systems commercial TRACERlab™ FX$_{FN}$ radiosynthesis module used for the synthesis of [^{18}F]FPEB is shown in the Figure 1. Automated synthesis involves the following: (1) azeotropic drying of [^{18}F]fluoride; (2) [^{18}F]fluorination; (3) in-line solvent exchange; and (4) HPLC purification, followed by solid-phase formulation of the final product. The synthesis module was operated in the following sequences with numerical references to the figure below.

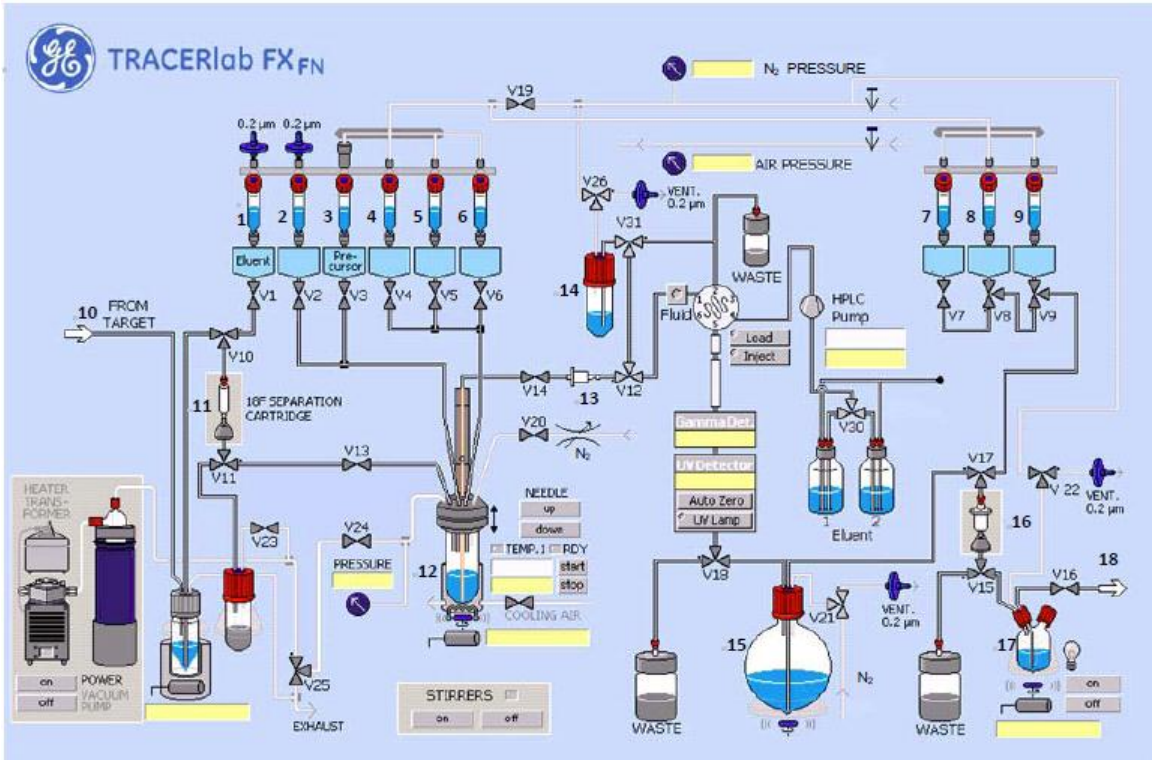


Figure S1: Schematic of the GE TRACERlab™ FX$_{FN}$ radiosynthesis module automated synthesis manifold for [^{18}F]FPEB

1. [^{18}F]Fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction using a GE cyclotron and delivered to the radiosynthesis module via 10. The [^{18}F]fluoride was quantitatively trapped on a QMA carbonate ion exchange solid phase extraction (SPE) light cartridge (Waters; activated with 6 mL of trace grade H_2O).
2. Automated synthesis began with the elution of resin-bound [^{18}F]fluoride using a solution (0.075M, 0.6 mL) of tetrabutylammonium hydrogencarbonate, pre-loaded into 1 and delivered to the reactor (12).

3. The reaction mixture (12) was dried azeotropically by addition of 1 mL anhydrous CH_3CN, pre-loaded into 4, at 85 °C under N_2 flow and vacuum over 8 min, then at 110 °C under N_2 flow and vacuum for 4 min.
4. After heating to 150 °C, nitro precursor (2 mg in 1.5 mL DMSO) pre-loaded into 3 was added to 12. The reactor was sealed *via* the closure of valve V13, V20 and V24 and the reaction mixture was heated for 15 min.
5. The reaction mixture was then cooled to 50 °C, vented *via* valves V24 and V25, and diluted with 10 mL of H_2O, pre-loaded into 5.
6. The contents of reaction vessel were delivered onto an Oasis® HLB Light SPE cartridge (13) (Waters; pre-activated with 5 mL EtOH followed by 10 mL H_2O) and washed with 5 mL of water from 6 to remove DMSO, unreacted ^{18}F-fluoride and other impurities. The crude reaction mixture was eluted from the cartridge (13) with 0.8 mL of CH_3CN from 2 into 14containing 1 mL of water. The contents of 14 were transferred to the HPLC loop *via* N_2 pressure *via* a fluid detector, injected onto a semi-preparative column (X-Select HSS T3, 250 × 10.00 mm, 5µ), and eluted with 45:55 CH_3CN/20 mM ammonium acetate by volume (pH 6) at a flow rate of 4 mL/min. The eluent was monitored by UV (λ = 254 nm) and radiochemical detectors connected in series.
7. A typical semi-preparative HPLC chromatogram is shown in Figure 2. The fraction containing the major radiochemical product (t_R = 19 min) was collected, via valve 18, into a large dilution vessel (15), which was preloaded with 20 mL of sterile water for injection (United States Pharmacopeia (USP); Hospira).

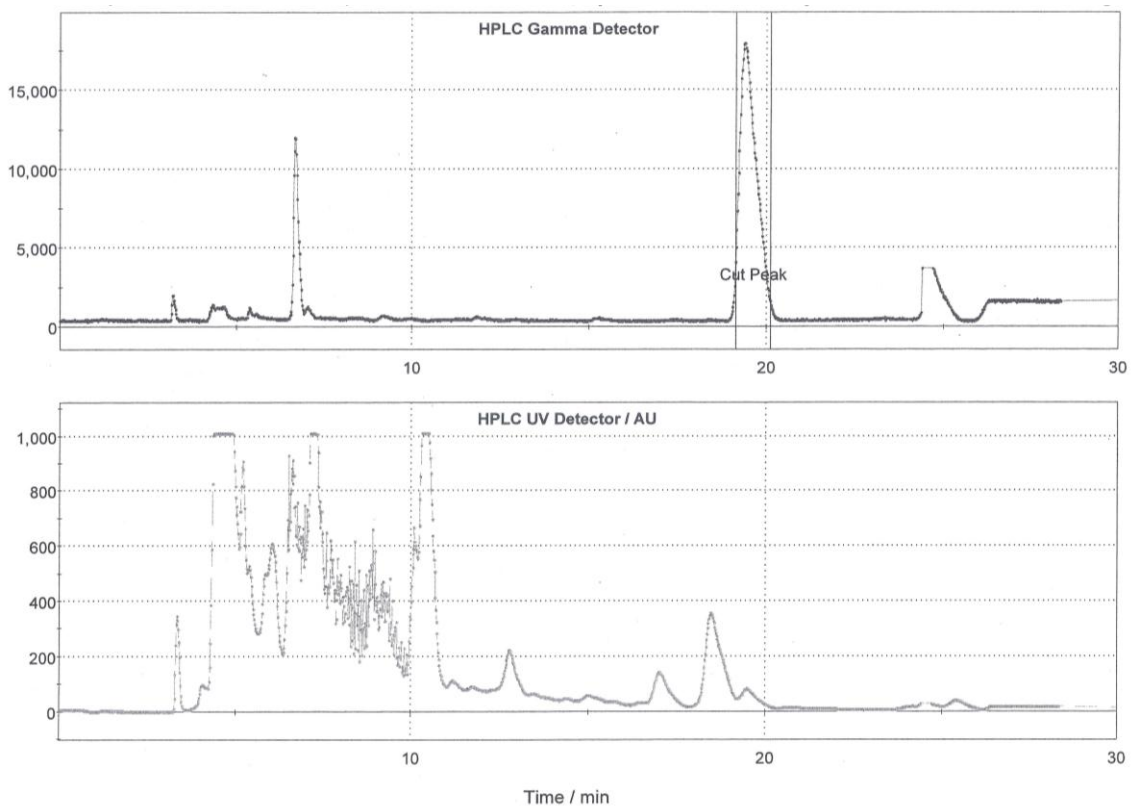


Figure S2: Semipreparative HPLC trace of a typical radiosynthesis of [^{18}F]FPEB

8. The diluted HPLC fraction was then loaded onto a C18 light SPE cartridge (16) (Waters; pre-activated with 5 mL EtOH followed by 10 mL H_2O).
9. The 16 was washed with 10 mL sterile water for injection, USP, preloaded into 7, to remove traces of salts, CH_3CN, and [^{18}F]fluoride.
10. The 16 was eluted with 1 mL dehydrated alcohol for injection, USP (Ethanol) preloaded into 8, into collection vial 17 followed by 10 mL 0.9% sodium chloride for injection, USP preloaded into 9.
11. The solution was transferred and passed through a 0.22 µm Millipore GV sterilizing filter (EMD Millipore) into a vented sterile 30 mL dose vial (Hospira).

Analyses of radioactive mixtures were performed by HPLC with an in-line UV ((λ = 254 nm) detector in series with a CsI PIN diode radioactivity detector. To determine the identity of [^{18}F]FPEB, aliquots of the formulated product were injected onto an analytical HPLC system using a Novapak C18 column, 150 × 4.6 mm, 4 µm and eluted with 45:55 EtOH/water at a flow rate of 1 mL/min, monitored at λ = 254 nm. The major radiochemical product was identified as [^{18}F]FPEB (t_R = 4.7 min; Figure 3). Uncorrected radiochemical yields of [^{18}F]FPEB were 7.0 ± 2.1% relative to starting [^{18}F]fluoride, and high specific activities were obtained in the final formulation (7.07 ± 1.38 Ci/µmol). Further characterization and validation of [^{18}F]FPEB was carried out as described in Quality Control section.

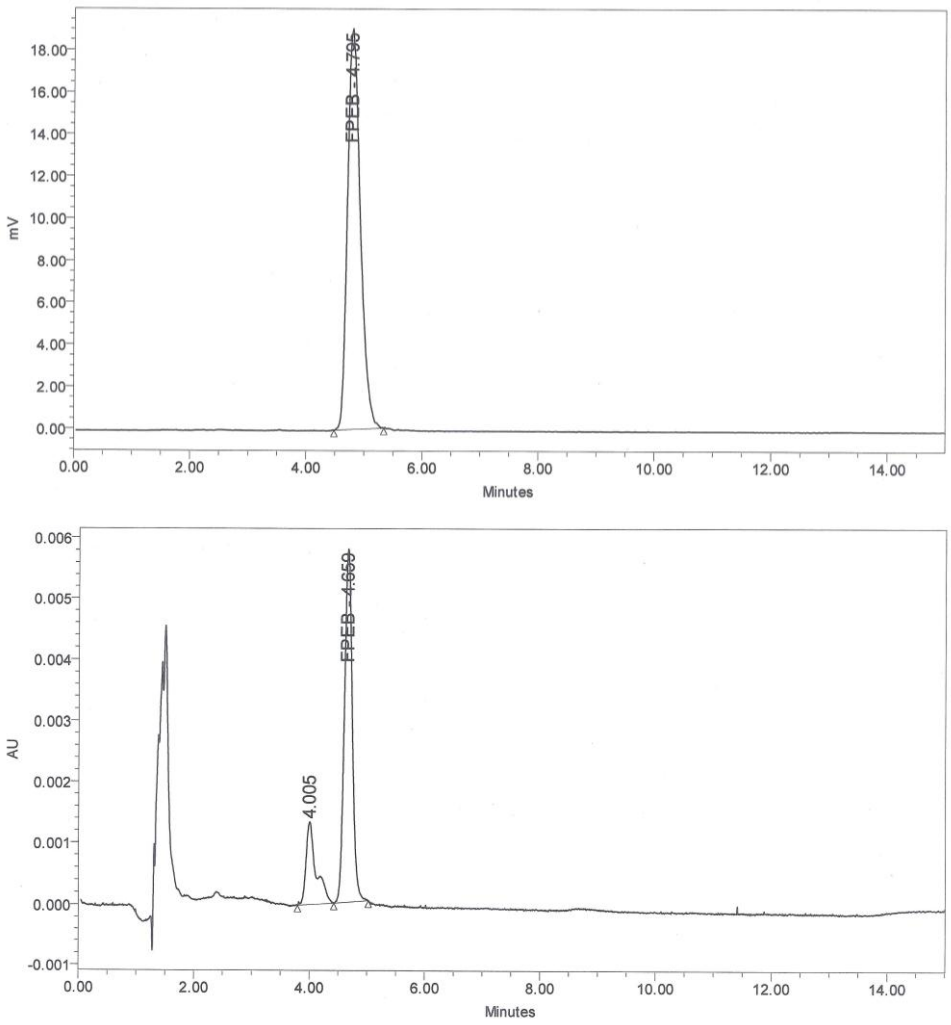


Figure S3: Analytical radioactive (top) and UV (bottom) HPLC traces for [^{18}F]FPEB

Quality control for [^{18}F]FPEB

The following tests were carried out in accordance with an MGH approved protocol, as per ICH and USP guidelines.

Visual Inspection: The [^{18}F]FPEB dose was clear, colorless, and free of particulate matter.

Radiochemical Identity, Radiochemical Purity, Injectable Mass and Specific Activity: To determine the identity of [^{18}F]FPEB, aliquots of the formulated product were injected onto an analytical HPLC system using a X-Select HSS T3, 150 × 4.6 mm, 3.5 μm and eluted with 45:55 EtOH/water at a flow rate of 1 mL/min, monitored at λ = 254 nm. After completion of the chromatograph, peaks on UV and radioactivity detector were integrated and the radiochemical and chemical purity were determined by the area of integration.

The major radiochemical product was identified as [^{18}F]FPEB (t_R ~4.7 min; Figure 3), followed by co-injection with the reference standard FPEB. The retention time of [^{18}F]FPEB was compared to that of the standard [^{19}F]FPEB and was within ±10% error. The radiochemical purity was >95%. Allowed injectable mass are as follows: ≤3.6 μg and ≤0.36 μg of unknown chemical impurities. Specific activity was determined using standard FPEB specific activity calibration curve. Specific activity must be ≥800 mCi per micromole at time of administration.

Residual Solvent Analysis: Residual solvent assay was performed to verify that residual solvents from in the synthesis and maintenance of the synthesis units are within acceptable limits. Gas chromatography (GC) was used to determine the solvent residue and the results met the following specifications.

DMSO (Class III) <5 mg/mL; Acetone (Class III) <5 mg/mL; Acetonitrile (Class II) <0.4 mg/mL; Ethanol (Class III) <10% v/v ± 10% (formulation agent)

pH Assay: The pH of [^{18}F]FPEB was determined by applying a few drops of the dose to pH indicator paper. Match the reference color and the pH value conformed to our release specifications (4.5-8.5).

Sterile Filter Integrity Test: Sterile filter integrity test was performed as per manufacturer specification and the pressure and was ≥ 50psi for the Millipore Millex GV 0.22 μm sterilizing filter.

Radionuclidic ID – photopeak and half-life: Measure the radioactivity of the formulated product at two separated time points. The half-life consistently met our release specifications (105-115 minutes).

Photopeak was determined based on the following protocol:

Introduce small amount of radioactivity of formulated product into gamma spectrometer. Record the spectrum and integrate the areas under the signals of the spectrum. The result was >99.5% emission @511 KeV, 1.022 MeV.

Endotoxin Analysis: Endotoxin analysis was performed on a Charles River Laboratories Endosafe PTS system using a 1:100 dilution. Doses contained ≤ 17.5 EU/mL.

Sterility Testing: Sterility testing was performed post-release and must be started within 30 hours from end of synthesis. [^{18}F]FPEB sample was inoculated into Trypitcase Soy Broth (TSB) and Fluid Thioglycollate Medium (FTM) media tubes. TSB tubes were incubated at 20-25°C and FTM tubes were incubated at 30-35°C for 14 days and must be free of culture growth after 14 days.

Automated synthesis of [^{18}F]FPEB by Advion Nanotek® method

A schematic diagram of the Advion NanoTek® microfluidic radiosynthesis module used for the synthesis of [^{18}F]FPEB is shown in Figure S4, The synthesis module was operated in the following sequences with numerical references to Figure S4:

1. [^{18}F]Fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction using a GE cyclotron (as described above) and delivered to the radiosynthesis module into vial 8. The [^{18}F]fluoride was quantitatively trapped on an ion exchange resin (F18 Trap & release cartridge, MP1, ORTG, Inc, Oakdale, TN, USA), which was activated with 6 mL of trace grade H_2O, and azeotropically dried three times with CH_3CN using standard NanoTek® fluoride drying macro.
2. The Precursor **1** (2 mg in 200 µL DMSO) and dried [^{18}F]*n*Bu$_4$NF, resolubilized in 250 µL DMSO, was flowed into the NanoTek® microfluidic 4 meter reactor (internal volume 32 µL) at 210 ºC using a total flow rate of 40 µL/min.
3. The ensuing reaction mixture was transferred into vial 11, pre-loaded with 10 mL of H_2O and fully mixed under a stream of nitrogen.
4. The contents of vial 11 were delivered onto an Oasis® HLB Light SPE cartridge on line 6 (Waters; pre-activated with 5 mL EtOH followed by 10 mL H_2O) and washed with 25 mL of water to remove DMSO, unreacted ^{18}F-fluoride and other impurities.
5. The crude reaction mixture was eluted from the cartridge with 1 mL of CH_3CN, followed by 3 mL of sterile water into vial 12.
6. The contents of vessel 12 were transferred to the HPLC loop (5 mL) of GE medical systems commercial TRACERlab™ FX_{FN} module, using the same HPLC conditions and formulation procedure for [^{18}F]FPEB.

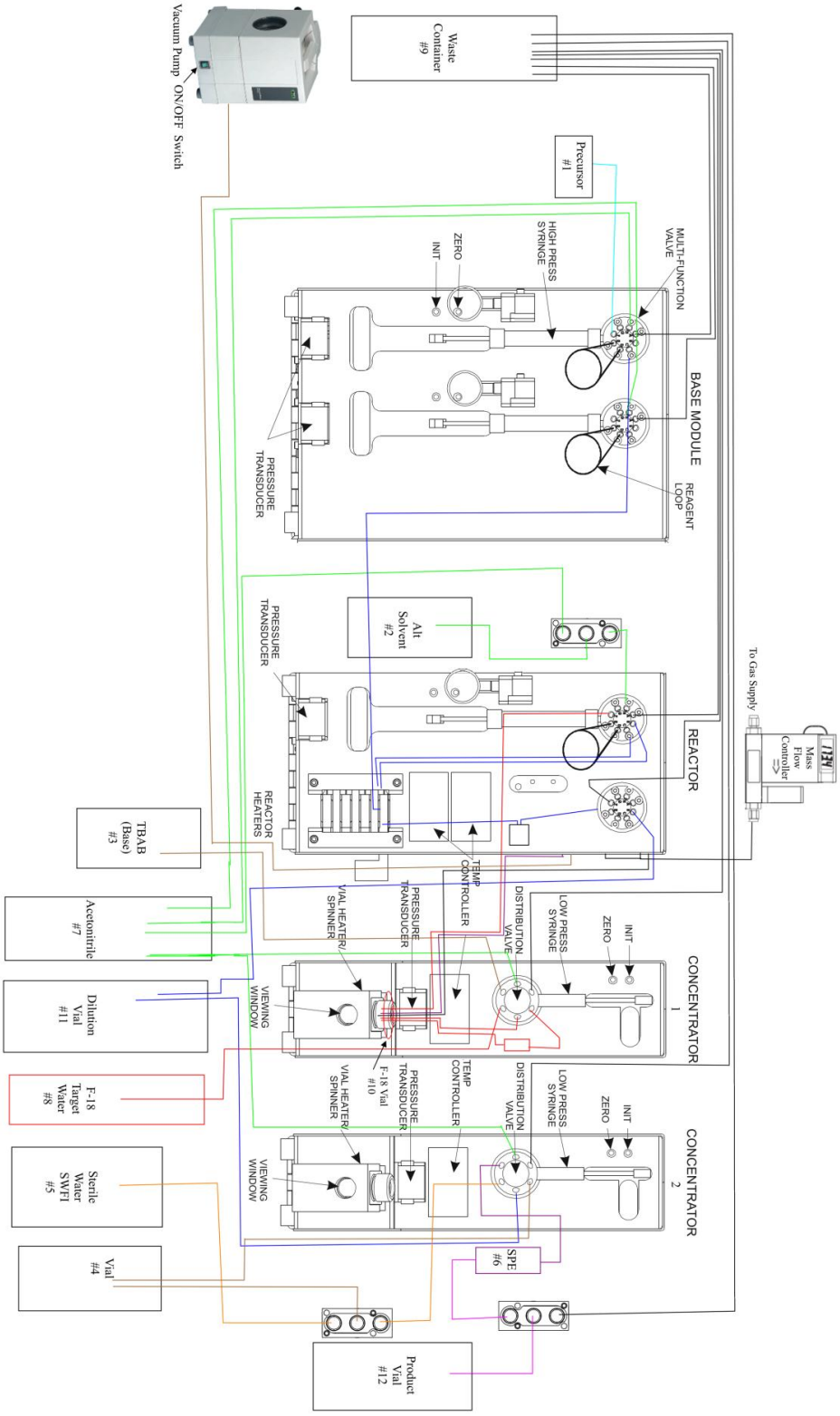


Figure S4. NanoTek® microfluidics plumbing diagram used for the synthesis of [^{18}F]FPEB

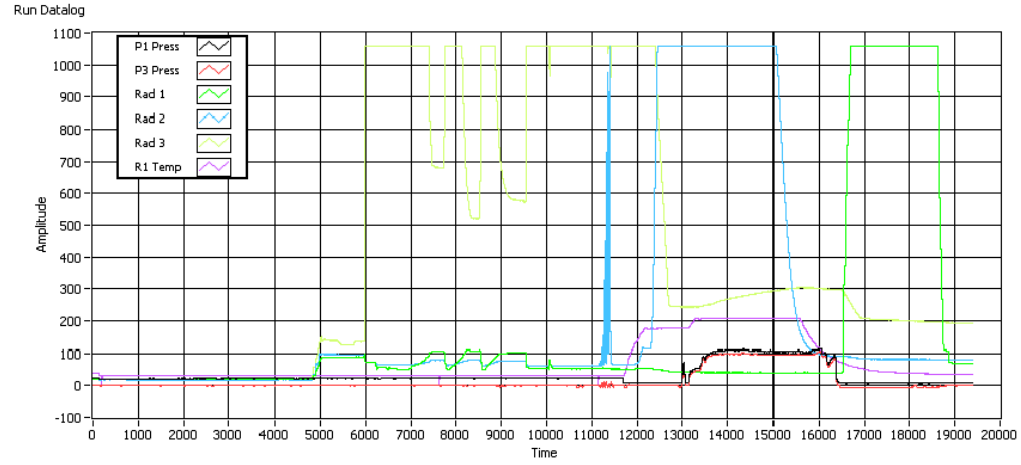
NanoTek [^{18}F]FPEB production log

Date	Company	Instr. SN	Operator	Run Compound	Plumbing	SOS	EOS
7/25/2013	MGH Hot Cell 2	MMLF0028	admin: Admin	FPEB	Custom		

C:\NanoTek Software\Sequencer Files\Fluoride Dry 3c_for_high_water_elution
C:\Documents and Settings\Advion\Desktop\UT2\UT Macros\addition of P3 alternate
C:\NanoTek Software\Sequencer Files\Mix_Fluoride_vial_through_loop_P3_faster
C:\NanoTek Software\Sequencer Files\FPEB Load Loops P1 P2 P3_and_lines_to_reactors_larger loops
C:\NanoTek Software\Sequencer Files\FPEB Reaction larger loops
C:\NanoTek Software\Sequencer Files\FPEB SepPak processing_V2

Name	Type	Formulation Date	Lot Number	Activity Tracking	Activity (uCi)	Time (HH:MM:SS PM)	Time Corrected	% Corrected
				Start			-	-

Notesred bown solution after HLB wash



ICP-MS analysis for trace metals

An automated cleaning procedure of microfluidic device was also performed (see ESI). To achieve this, we used three different solvents, including sterile water, ethanol and acetonitrile. Following the cleaning cycle, a non-radioactive run (no precursor; DMSO only) was carried out through the synthesis process. The solution was analysed by analytical HPLC and confirmed that residual **1** or FPEB were not present. A further analysis by ICP-MS (Galbraith laboratories, TN, USA) to test for 60 common metals showed that all impurities, if any, were significantly below regulatory limits.

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Laboratory Report

Report prepared for:
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Sample: FPEB 4-29-2013
Lab ID: 2013-U-4711 **Received:** 2013-07-25

Analysis	Method	Result	Basis	Sample Amount Used	Date (Time)
400: ICP-MS Screen	GLI Procedure ME-31	See Attachment	As Received	258.11 mg	2013-07-29

Signatures:

Created By:	Debbie.S.Robertson	2013-08-01T20:40:13.733-04:00
Published By:	Debbie.S.Robertson	2013-08-01T20:40:34.64-04:00

- Physical signatures are on file.
- "Published By" signature indicates authorized release of data.

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GALBRAITH LABORATORIES, INC.

Mass Spec Semi-Quantitative Screen

GLI Sample ID U-4711 Sample Amt 258.11 mg
Data Sheet Final Volume 50 mL
Reference 5003 Dilution Factor 5

Element	Result	Element	Result
Lithium	<2 ppm	Cadmium	<2 ppm
Beryllium	<2 ppm	Indium	<2 ppm
Boron	4 ppm	Tin	<2 ppm
Sodium	<20 ppm	Antimony	<2 ppm
Magnesium	4 ppm	Tellurium	<2 ppm
Aluminum	19 ppm	Cesium	<2 ppm
Phosphorus	<20 ppm	Barium	<2 ppm
Potassium	<20 ppm	Lanthanum	<2 ppm
Calcium	N/A	Cerium	<2 ppm
Scandium	<2 ppm	Praseodymium	<2 ppm
Titanium	<2 ppm	Neodymium	<2 ppm
Vanadium	<2 ppm	Samarium	<2 ppm
Chromium	<2 ppm	Europium	<2 ppm
Manganese	<2 ppm	Gadolinium	<2 ppm
Iron	<20 ppm	Terbium	<2 ppm
Cobalt	<2 ppm	Dysprosium	<2 ppm
Nickel	<2 ppm	Holmium	<2 ppm
Copper	<2 ppm	Erbium	<2 ppm
Zinc	<2 ppm	Thulium	<2 ppm
Gallium	<2 ppm	Ytterbium	<2 ppm
Germanium	<2 ppm	Lutetium	<2 ppm
Arsenic	<2 ppm	Hafnium	<2 ppm
Selenium	<2 ppm	Tantalum	<2 ppm
Rubidium	<2 ppm	Tungsten	<2 ppm
Strontium	<2 ppm	Rhenium	<2 ppm
Yttrium	<2 ppm	Iridium	<2 ppm
Zirconium	<2 ppm	Platinum	<2 ppm
Niobium	<2 ppm	Gold	<20 ppm
Molybdenum	<2 ppm	Thallium	3 ppm
Ruthenium	<2 ppm	Lead	<2 ppm
Rhodium	<2 ppm	Bismuth	<2 ppm
Palladium	<2 ppm	Thorium	<2 ppm
Silver	<2 ppm	Uranium	N/A

Note: The above results are semi-quantitative. They may contain up to 50% relative error.

Mailing: P.O. Box 51610 | Knoxville, TN 37950-1610
Toll Free: 1.877.449.8797
www.galbraith.com
Shipping: 2323 Sycamore Dr. | Knoxville, TN 37921-1700
Fax: 865.546.7209

NanoTek [^{18}F]FPEB reaction macro sequence

1_Fluoride Dry 3b_for_high_water_elution
2_addition of P3 alternate
3_Mix_Fluoride_vial_through_loop_P3_faster
4_FPEB Load Loops P1 P2 P3_and_lines_to_reactors_larger loops
5_FPEB Reaction larger loops
6_FPEB SepPak processing_V2

Detailed commands:

1_Fluoride Dry 3b_for_high_water_elution

0 /2u2R Make Sure P3 is on ACN
100 /3o1V3200A0o8V3200A24000o1V3200A0R Clean P3 to make sure it has only ACN
100 /7U3R DH-3 DO Contact Open
4000 /7u3R DH-3 DO Contact Closed
2000 L37020001250052 L3602000300004E Set Temp to 125C
0 Wait When Fluoride is ready press OK
10000 /5go1V3200A12000M2000o5V1200A0M500G6go3V3200A6000M1000o1A0M500A8000o5V1200A0M500G2go6V6400A12000M1000o4A0G4R CE: Load Target Water
180000 AO-2 1.5 User 1 AO 5.0 VDC reduced from 2.5
5000 /7U1R Open Gas Valve
5000 /7U2M5000U1M5000u2R G: Turn on Nitrogen
4000 /5o2V800P1200o4V3200A0R Fill Kryptofix line and send to waste
20000 /5o2V2000A7200M1500go5V400D1500M60000G4o2V2000A0R CE: Add 450 uL K222/K2CO3
280000 /5o3V2000A2000M2000o5V75D600o6V75D1200o2V2000A0o4V800P1200o5V40D1200R CE: Add MeCN
15000 /3o8V3200A4800M1000o6V300D2400M500o1V2000A0M500o2R P3: Add MeCN
180000 /3o1R Delay 2.5 min
2000 L37020001050052 L3602000300004E Set Temp to 105C
15000 /5o3V2000A2000M2000o5V75D600o6V75D1200o2V2000A0o4V800P1200o5V40D1200R CE: Add MeCN
15000 /3o8V3200A4800M1000o6V300D2400M500o1V2000A0M500o2R P3: Add MeCN
180000 /5o3V2000A2000M2000o5V75D600o6V75D1200o2V2000A0o4V800P1200o5V40D1200R CE: Add MeCN
15000 /3o8V3200A4800M1000o6V300D2400M500o1V2000A0M500o2R P3: Add MeCN
210000 /5o5V3200A12000o5V800A6000M30000o6V800A0R Flush the lines out to remove any remaining solvent from the lines - minimize splashing of activity on walls
120000 /7U1R G: Turn on Nitrogen
2000 L37020000260054 L3602000300004E CE: Set Heater to 26oC
30000 /1U3R Vac Pump Control Turn ON Vac Pump
2000 /7U3R Switch Vessel to Vacuum

2000 /7u1R Turn Off Gas
90000 /7U1R Turn Gas ON to Pressurize Vessel
5000 /1u3R Vac Pump Control Turn OFF Vac Pump
2000 /7u3R Switch Vessel to Atmosphere
5000 /7u1R Turn Off Gas
1000 AO-2 0.0 Turn Gas off at MFC
2000 L37020000260054 L3602000300004E CE: Set Heater to 26oC

2_addition of P3 alternate

0 /3o1V4800A0R Empty Syringe to waste
15000 /2U2R Select Alternate solvent
5000 /3o6V4000P6000o1V4000A0o8V3200A48000o1V3200A0o8V3200A19800o6V360D19800R "Clean Syringe with Alternate solvent and use the alternate for all Discovery Operations, dissolve in 450 ul"
120000 /3M0R Fluoride now in Alternate Solvent and system ready to use alternate solvent

3_Mix_Fluoride_vial_through_loop_P3_faster

0 L3702000100004D L3602000300004E Set Concentrator to 100C to aid in dissolving fluoride
1000 /3go4V1600A14400o4V3200A0G5o8V3200A4128M2000o4V3200A0R Mix solvent 5 times and then flush solvent line with 100 ul of solvent to prepare for discovery
120000 L37020000250053 L3602000300004E Set concentrator back to RT

4_FPEB Load Loops P1 P2 P3_and_lines_to_reactors_larger loops

0 /7o6R Make sure we are on Waste line from Distribution Hub
10 /2U2R Make sure we are on DMSO
10 L33020001800051 Pre-heat Reactor 2 to 180C
100 L3202000180004D Pre-heat Reactor 1 to 180C
1000 /1o1V6400A0o8V6400A48000o1V6400A0R Empty Pump 1 and Flush Syringe with Acetonitrile to Waste
1000 /3o1V3200A0o8V3200A48000o1V3200A0R Empty P3 Syringe and clean with Acetonitrile
50000 /1o6V96P3936o1V1600A0R Fill Reagent line with Precursor and empty syringe to waste
100 /3o6V72A4113o1V3200A0R Fill Reagent line with Fluoride and empty syringe to waste
95000 /1o4V640P19958R Fill Precursor Loop with Precursor in one minute
1000 /3o4V160A19090R Fill Fluoride Loop with Fluoride
145000 /1o1V3200A0o8V6400A48000o5V80D2784o8V6400A48000R Empty P1 Syringe and fill with Acetonitrile push to reactor and refill syringe
100 /3o1V3200A0o8V3200A48000o5gv700D1M10G816o8V3200A48000R Empty P3 Syringe and fill with Acetonitrile push to reactor and refill syringe

5_FPEB Reaction larger loops

0 /7U2R Turn N2 gas to concentrator 2 ON
100 AO-2 0.2 Set gas flow to 100 ml/min to mix solution
10 L33020002000051 Bring Reactor 2 up to 200 to aid in rapid heating of reactor 2
10 L32020002100050 Bring the reaction temp up to 210C
0 /1o5gv800D1M17G24768R Push 257.9 ul out of P1 at 20 ul/min
100 /3o5gv800D1M17G23856o5gv700D1M27G1920R Push 497 ul out of P3 at 40 ul / min
20000 /7o2R Switch Distribution hub from Waste to HPLC
600000 L3202000025004E Set reactor back to RT
100 L3302000025004F Set reactor 2 to RT

6_FPEB SepPak processing_V2

0 /6o4V4000A0R Make sure syringe is empty
15000 AO-2 0.25 Turn on the gas to mix the DMSO and water solution
100 /7U2R Turn on the Gas to Concentrator 2
100 /6go1V4000A12000o4V4000A0G2R Rinse syringe with water
10000 /1u3R Make sure we are on Water
100 /2u3R Make sure we are set to waste port on SPE
100 AO-2 0.25 Turn off gas at MFC
100 /7u2R Shut Gas valve
10000 /6go6V4000A12000o2V2000A0G11ggo1V4000A12000o6V4000A0G5go6V4000A12000o2V4000A0G5G5R "Push diluted solution over SPE, rinse vessel with water and push over SPE"
500000 /6go3V4000A12000o4V4000A0G2o3V4000A12000R "Clean the syringe with acetonitrile, and fill with ACN"
25000 /1U3R Switch Valve to elute FPEB to vial with mobile phase
100 /6o3V4000A12000o2V400D6000o2V200D6000go1V4000A12000o4V4000A0G3go1V4000A12000o2V2000A0G3R Flush SPE with 1 ml of Acetonitrile then flush with 2 ml of Water
120000 /1u3R Switch back to Waste

NanoTek [^{18}F]FPEB cleaning macro sequence

1_Master Clean_Conc1_and_2_FPEB_water

2_Master Clean_Conc1_and_2_FPEB_Ethanol

3_Master Clean_Conc1_and_2_FPEB_Acetonitrile

Detailed commands:

1_Master Clean_Conc1_and_2_FPEB_water

0 Wait Remove reaction vessel, place cap in container to collect waste and place lines for fluoride and Kryptofix in waste containers. Remove fluoride trap and replace with length of tubing. Replace Acetonitrile bottle with Water and make sure Waste container(s) is (are) Empty. Make sure dilution vial is replaced with a clean vial, remove HLB cartridge and connect leur fittings together. Make Sure concentrator 2 Processing vial is Empty and product elivery line is in Waste container Press OK when Ready

100 /2u2R Make sure we are on Acetonitrile

100 /6o1V3200A0R Con. 2 Move

2000 /5o1V3200A0R Con.1 Move

2000 /3o1V3200A0R P3 Move

2000 /2o1V6400A0R P2 Move

2000 /1o1V6400A0R P1 Move

2000 L39020000250055 L38020003000050 Set all temps to 25C

2000 L37020000250053 L3602000300004E Set all temps to 25C

2000 L35020000250051 Set all temps to 25C

2000 L34020000250050 Set all temps to 25C

2000 L3302000100004F Set all temps to 25C

2000 L3202000100004E Set all temps to 25C

3000 /1o1V3200A0o8A48000M1000o1A0o8A48000M1000o4V1600A0o8V3200A48000M1000o3V400A0R Zero 1 Fill

0 /7o2R Hub to FPEB dilution vial

5000 /3o1V3200A0o8A48000M1000o1A0o8A48000M1000o4V1600A0o8V3200A48000o3V120A24000o2V80A0R Zero 3 Fill and System Sweep

2000 /5o3V3200A12000M1000o1A0o3A12000M1000o1A0o3A12000M1000o2A0o3A12000M1000o2A0R Clean A&B

50000 /5o3A12000M1000o4A0o3A12000M1000o4A0o3A12000M1000o6A0o3A12000M1000o6A0R Clean D&F

50000 /5o3A12000M1000o5A0o3A12000M1000o5A0o3A12000M1000o5A0RClean E

100 /6go3V4000A12000o4V4000A0G2R Clean syringe with acetonitrile

18000 /6go3V4000A12000o6V4000A0G2R Clean Port F

18000 /6go3V4000A12000o5V4000A0G2R Clean Port E line to HPLC loop

18000 /6go3V4000A12000o2V4000A0G2R Clean SPE to Waste

18000 /1U3R Switch SPE to Product collection vial

100 /6go3V4000A12000o2V4000A0G2R Clean SPE to product vial

25000 /1u3R Switch back to waste line

100 /2U3R Switch to reformulation vial
100 /6go3V4000A12000o1V4000A0G2R Wash reformulation line with ACN
30000 /2u3R switch back to water
100 /6go1V4000A12000o4V4000A0G2R Clean syringe with water
25000 /6go1V4000A12000o5V4000A0G2R Clean port E with water
25000 /6go1V4000A12000o6V4000A0G2R Clean port F to waste
18000 /6go1V4000A12000o2V4000A0G2R Clean port B to waste through SPE line
18000 /1U3R Switch SPE to reformulation line
1000 /1o2V61P0R Change to a plugged port
2000 /2o2V61P0R Change to a plugged port
100 /6go1V4000A12000o2V4000A0G2R Clean Port B and SPE to reformulation
18000 /1u3R Switch SPE to waste again
100 /6o1V4000A12000R
10000 /2U3R Switch to Reformulation vial
100 /6o1V4000A0R
10000 /2u3R Back to water
100 /6o1V4000A12000R
10000 /2U3R
100 /6o1V4000A0R
10000 /2u3R

2_Master Clean_Conc1_and_2_FPEB_Ethanol

0 Wait Replace Water container with Ethanol, empty waste containers. Press OK to continue
100 /2u2R Make sure we are on Acetonitrile
100 /6o1V3200A0R Con. 2 Move
2000 /5o1V3200A0R Con.1 Move
2000 /3o1V3200A0R P3 Move
2000 /2o1V6400A0R P2 Move
2000 /1o1V6400A0R P1 Move
16000 /1o1V3200A0o8A48000M1000o1A0o8A48000M1000o4V1600A0o8V3200A48000M1000o3V400A0R Zero 1 Fill
0 /7o2R Hub to FPEB dilution vial
5000 /3o1V3200A0o8A48000M1000o1A0o8A48000M1000o4V1600A0o8V3200A48000o3V120A24000o2V80A0R Zero 3 Fill and System Sweep
2000 /5o3V3200A12000M1000o1A0o3A12000M1000o1A0o3A12000M1000o2A0o3A12000M1000o2A0R Clean A&B
50000 /5o3A12000M1000o4A0o3A12000M1000o4A0o3A12000M1000o6A0o3A12000M1000o6A0R Clean D&F
50000 /5o3A12000M1000o5A0o3A12000M1000o5A0o3A12000M1000o5A0RClean E
100 /6go3V4000A12000o4V4000A0G2R Clean syringe with acetonitrile

18000 /6go3V4000A12000o6V4000A0G2R Clean Port F
18000 /6go3V4000A12000o5V4000A0G2R Clean Port E line to HPLC loop
18000 /6go3V4000A12000o2V4000A0G2R Clean SPE to Waste
18000 /1U3R Switch SPE to Product collection vial
100 /6go3V4000A12000o2V4000A0G2R Clean SPE to product vial
25000 /1u3R Switch back to waste line
100 /2U3R Switch to reformulation vial
100 /6go3V4000A12000o1V4000A0G2R Wash reformulation line with ACN
30000 /2u3R switch back to water
100 /6go1V4000A12000o4V4000A0G2R Clean syringe with water
25000 /6go1V4000A12000o5V4000A0G2R Clean port E with water
25000 /6go1V4000A12000o6V4000A0G2R Clean port F to waste
18000 /6go1V4000A12000o2V4000A0G2R Clean port B to waste through SPE line
18000 /1U3R Switch SPE to reformulation line
1000 /1o2V61P0R Change to a plugged port
2000 /2o2V61P0R Change to a plugged port
100 /6go1V4000A12000o2V4000A0G2R Clean Port B and SPE to reformulation
18000 /1u3R Switch SPE to waste again
100 /6o1V4000A12000R
10000 /2U3R Switch to Reformulation vial
100 /6o1V4000A0R
10000 /2u3R Back to water
100 /6o1V4000A12000R
10000 /2U3R
100 /6o1V4000A0R
10000 /2u3R

3_Master Clean_Conc1_and_2_FPEB_Acetonitrile

0 Wait Replace Acetone container with Acetonitrile, empty waste containers. Press OK to continue
100 /2u2R Make sure we are on Acetonitrile
100 /6o1V3200A0R Con. 2 Move
2000 /5o1V3200A0R Con.1 Move
2000 /3o1V3200A0R P3 Move
2000 /2o1V6400A0R P2 Move
2000 /1o1V6400A0R P1 Move
16000 /1o1V3200A0o8A48000M1000o1A0o8A48000M1000o4V1600A0o8V3200A48000M1000o3V400A0R Zero 1 Fill
0 /7o2R Hub to FPEB dilution vial
5000 /3o1V3200A0o8A48000M1000o1A0o8A48000M1000o4V1600A0o8V3200A48000o3V120A24000o2V80A0R Zero 3 Fill and System Sweep

2000
/5o3V3200A12000M1000o1A0o3A12000M1000o1A0o3A12000M1000o2A0o3A12000
M1000o2A0R Clean A&B
50000
/5o3A12000M1000o4A0o3A12000M1000o4A0o3A12000M1000o6A0o3A12000M1000
o6A0R Clean D&F
50000 /5o3A12000M1000o5A0o3A12000M1000o5A0o3A12000M1000o5A0RClean E
100 /6go3V4000A12000o4V4000A0G2R Clean syringe with acetonitrile
18000 /6go3V4000A12000o6V4000A0G2R Clean Port F
18000 /6go3V4000A12000o5V4000A0G2R Clean Port E line to HPLC loop
18000 /6go3V4000A12000o2V4000A0G2R Clean SPE to Waste
18000 /1U3R Switch SPE to Product collection vial
100 /6go3V4000A12000o2V4000A0G2R Clean SPE to product vial
25000 /1u3R Switch back to waste line
100 /2U3R Switch to reformulation vial
100 /6go3V4000A12000o1V4000A0G2R Wash reformulation line with ACN
30000 /2u3R switch back to water
100 /6go1V4000A12000o4V4000A0G2R Clean syringe with water
25000 /6go1V4000A12000o5V4000A0G2R Clean port E with water
25000 /6go1V4000A12000o6V4000A0G2R Clean port F to waste
18000 /6go1V4000A12000o2V4000A0G2R Clean port B to waste through SPE line
18000 /1U3R Switch SPE to reformulation line
1000 /1o2V61P0R Change to a plugged port
2000 /2o2V61P0R Change to a plugged port
100 /6go1V4000A12000o2V4000A0G2R Clean Port B and SPE to reformulation
18000 /1u3R Switch SPE to waste again
100 /6o1V4000A12000R
10000 /2U3R Switch to Reformulation vial
100 /6o1V4000A0R
10000 /2u3R Back to water
100 /6o1V4000A12000R
10000 /2U3R
100 /6o1V4000A0R
10000 /2u3R
10000 L3302000025004F
1000 L3202000025004E