[^{18}F]FBR for Injection:
Standard Operating Procedures

PET Radiopharmaceutical Sciences Section,
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Date of review: Draft 8/17/2009

List of SOPs for *[^{18}F]FBR for Injection*

Document Title	Number
Preparation of HPLC Mobile Phases	SOP # GP 101
Preparation of Stock Solution of Kryptofix 2.2.2[1] and Potassium Carbonate	SOP # GP102
Cleaning Procedures for Radiosynthesis Apparatus	SOP # GP103
Cleaning Procedure for Radiochemistry Glassware	SOP # GP104
Production of *[^{18}F]FBR for Injection*. Part 1: Preliminary Procedures	SOP # MP 201
Production of *[^{18}F]FBR for Injection.* Part 2: Synthesis and Formulation	SOP # MP 202
Release of *[^{18}F]FBR for Injection*	SOP # QA301
Sampling and Quality Control Procedures for *[^{18}F]FBR for Injection*	SOP # QA302
Analysis of Organic Residues in by Gas Chromatography	SOP # QA 303
Analytical HPLC Quality Control Method	SOP # QA 304
FBR Precursor and Standard Acceptance Criteria	SOP # QA 305
Standard HPLC Calibration Curve of Reference FBR	SOP # QA 306
Materials, Instruments, and Equipment	Appendix A
Synthia Methods and Positions	Appendix B
Calculations Worksheet	Appendix C
Representative Chromatograms	Appendix D
Representative, FBR Reference Material	Appendix E
Representative, Br-FBR Precursor	Appendix F

[1] 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8] hexacosane.

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Date of review: Draft 8/17/2009

SOP # GP101

[^{18}F]*FBR for Injection*: Preparation of HPLC Mobile Phases

Approved by: _____ Initials;_____ Date:_____

Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: To prepare mobile phase required for the HPLC Quality Control analysis of *[^{18}F]FBR for Injection.*

Analytical Mobile Phase

The following instructions are for the preparation of 1L of solution. Quantities may be scaled according to the volume required.

1. One liter of analytical HPLC mobile phase is prepared by adding 0.63 g ammonium formate to one (1) liter 60% v/v acetonitrile in HPLC grade water.
2. Mix to dissolve. Filter through a 0.45 μm nylon filter (Phenomenex AFO-054 or equivalent).
3. Transfer the filtered solvent into a clean HPLC reservoir bottle. Label the vessel with description of contents, date of preparation, and expiration date. The buffer may be used for one week after the preparation date, provided that it is tightly capped and stored at room temperature when not in use.

Preparative Mobile Phase

1. HPLC grade acetonitrile and HPLC water are used without modification.
   1.1. Install the HPLC grade water on the "A" pump.
   1.2. Install the HPLC grade acetonitrile on the "B" pump.
2. Filtering is required for bottles previously opened.

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Date of review: Draft 8/17/2009

SOP # GP102

Preparation of Stock Solution of Kryptofix 2.2.2[2] and Potassium Carbonate

Approved by: _____ Initials;_____ Date:_____

Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: To prepare a stock solution of Kryptofix 2.2.2 in aqueous potassium carbonate

The following instructions are for the preparation of 2 mL of solution. Quantities may be scaled according to the volume required.

1. Weigh 10 ± 0.2 mg of potassium carbonate (Sigma Aldrich, anhydrous, 99.99%) to a vessel of appropriate size. Add deionized water (0.2 mL) and mix to dissolve.
2. Weigh 100 ± 1 mg of Kryptofix 2.2.2 (Sigma Aldrich anhydrous; 98%) and transfer to the vessel.
3. Add anhydrous acetonitrile (1.8 mL) and mix to dissolve until a homogeneous solution is obtained.
4. Transfer the stock solution to a tightly sealed appropriate storage vessel
5. Label with description, expiration date (two weeks), and constituent component lot numbers and quantities.
6. Store at 0 – 8 °C.

Note: 100 µL of solution contains 0.5 mg potassium carbonate and 5 mg Kryptofix 2.2.2.

[2] 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8] hexacosane.

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Date of review: Draft 8/17/2009

SOP # GP103

Cleaning Procedures for Radiosynthesis Apparatus

Approved by: _____ Initials;_____ Date:_____

Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: Cleaning Procedures for the Synthia Radiosynthesis apparatus

1. Pre-synthesis
   - 1.1. Set up the vials and reagents according to the instructions found in SOP# MP 201, section1.7, Reagent Setup.
   - 1.2. Fill the syringe reservoir with HPLC grade water.
   - 1.3. Run the Synthia recipe CLEANING.
   - 1.4. Formulation Line
     - 1.4.1. The formulation line must be cleaned manually. Push a minimum of 5 mL ethanol through the line and blow dry with the house air supply.
   - 1.5. Collection line
     - 1.5.1. Run 100% acetonitrile through the prep HPLC system. This should be done with only a span of tubing and no column in-line.
     - 1.5.2. Use V 41 using Synthia's manual mode to flush at least 5 mL of acetonitrile through the collection line.
2. Post synthesis
   - 2.1. Column
     - 2.1.1. After each synthesis the preparative and analytical columns are washed with 100 % acetonitrile for a minimum of 20 column volumes.
   - 2.2. Synthia Apparatus (should be performed when residual radioactivity is below detectable levels).
     - 2.2.1. Fill the syringe reservoir with 70% aqueous ethanol.
     - 2.2.2. Fill F3 and F7 with absolute ethanol.
     - 2.2.3. Remove the HPLC column and replace it with a span of tubing. Run 100% acetonitrile or absolute ethanol through both pumps.
     - 2.2.4. Run the Synthia recipe CLEANING.

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Date of review: Draft 8/17/2009

SOP # GP104

Cleaning Procedure for Radiochemistry Glassware

Approved by: _____ Initials;_____ Date:_____

Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: Cleaning procedures for production glassware

Procedure

1. Rinse each item with water, acetone, and/or other appropriate organic solvent as required to remove residue.
2. Bathe the glassware in an aqueous 2% solution of Liqui-Nox (Valconox).
   2.1. Bring the solution to a low boil for 5–10 minutes minimum. Allow to cool.
   2.2. Rinse glassware at least 3 times with Millipore water.
3. Carefully check each item. Repeat cleaning steps if required. Gentle scrubbing may be employed.
4. Place glassware in an oven at 70 ºC (minimum) and allow to dry completely.

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Date of review: Draft 8/17/2009

SOP # MP201

Production of *[^{18}F]FBR for Injection*. Part 1: Preliminary Procedures

Approved by: _____ Initials;_____ Date:_____

Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: Setup instruments and apparatus for production of *[^{18}F]FBR for Injection*.

1. Setup
   - 1.1. Install a clean 2 mL conical vial with 20 mm, 10/90 mil PFTE septa (e.g.; Alltech Cat. No. 95303) in the microwave oven. Clamp the vial in place.
   - 1.2. Install a clean 5 mL conical vial with 20 mm, 10/50 mil PFTE septa (e.g.; Alltech Cat. No. 95302) in the thermal oven. Clamp the vial in place.
   - 1.3. Install clean nitrogen and vent needles at the thermal and microwave ovens. Ensure the needles at the thermal oven are clamped in place.
   - 1.4. Verify the ionization chamber by measuring the ^{57}Co and ^{137}Cs standards. Compare the measurements to the expected measurements for the day's date. Record the measurements in the Quality Control Record and in the tracking spreadsheet. Report any measurement that is not within ± 5% of the expected measurement.
   - 1.5. Verify the portable balance by measuring the mass of a 10 g NIST traceable calibrated standard weight. The weight should measure 10 ± 0.1g. Record the measurement on the Master Batch Record.
   - 1.6. Cleaning formulation and collection lines
     - 1.6.1. Clean the formulation and collection lines according to SOP# GP103, section 1.6.
   - 1.7. Reagent Setup
     - 1.7.1. Set up all vials required by Table 1 using the Diagram in Figure 1 for guidance.[3]

[3] Note: The positions depicted are three dimensional when the vial is included. Using a vial other than specified will require recalibration of the robot arm position for the new vial type. A more complete discussion may be found in Appendix B.

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Figure 1: Diagram of Synthia Deck Layout

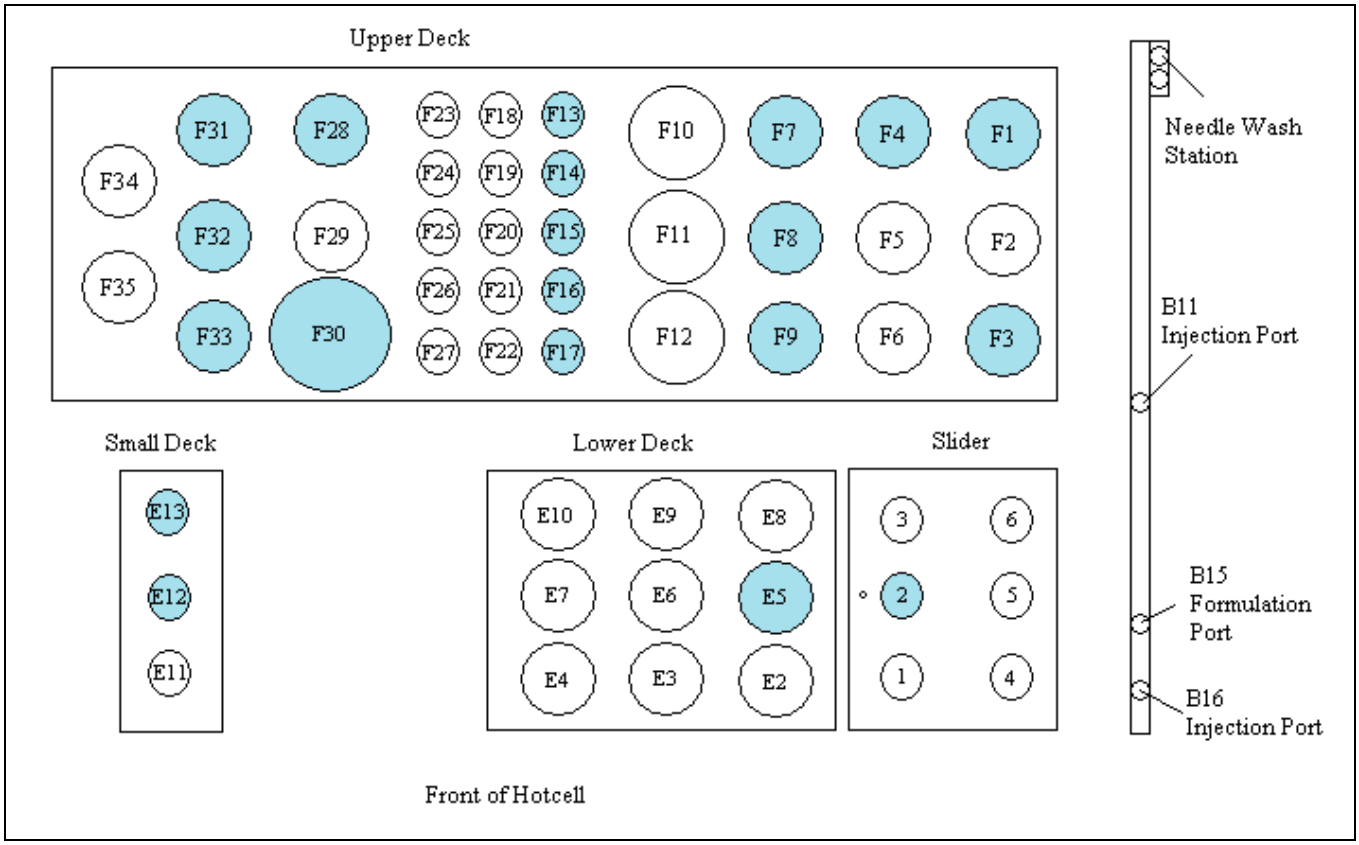

Table 1

Position	Tube Type	Solvent/ Material	Position	Tube Type	Solvent/ Material
F1	10 mL round bottom	Empty/ Waste	F17	High Recovery autosampler vial w/ cap	1 mL ethanol, injection USP
F3	10 mL round bottom	10 mL Acetonitrile	F28	5 mL serum finish	5 mL Absolute Ethanol
F4	10 mL round bottom	10 mL Absolute Ethanol	F30	Large	13 mL HPLC grade water
F7	10 mL round bottom	10 mL Acetonitrile	F31	10 mL round bottom	10 mL Absolute Ethanol
F8	10 mL round bottom	10 mL Acetonitrile	F32	10 mL round bottom	10 mL Absolute Ethanol
F9	10 mL round bottom	10 mL Sterile Saline, injection USP	F33	2 mL thick walled conical	Activity from CC
F13	High Recovery autosampler vial w/ cap	Precursor in MeCN 1 mg/ mL	E5	1 mL thick wall conical with serum finish	Empty
F14	1.7 mL Autosampler vial	10 mL Absolute Ethanol	E12	Flat bottom 3 mL	3 mL Absolute Ethanol
F15	1.7 mL Autosampler vial	10 mL Absolute Ethanol	E13	Flat bottom 3 mL	3 mL Absolute Ethanol
F16	High Recovery autosampler vial	1 mL Acetonitrile	Slider 2	SPE Cartridge	Activated

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1.7.2. Place the required solvents and reagents as indicated in Table 1. in the corresponding vials.

1.7.2.1. Activate the SPE cartridge with a minimum of 3 mL ethanol followed by a minimum of 3 mL water and place into slider position 2.

1.7.2.2. Weigh 0.85 ± 0.1mg precursor in a tared, high recovery auto-sampler vial. Dissolve in acetonitrile to give a 1 mg/mL (nominal) solution. Cap with a pierce-able auto-sampler cap and place in position F13.

1.7.3. Empty the HPLC waste and the waste container under the needle wash.

1.7.4. Empty and refill the syringe reservoir with HPLC grade water.

1.8. Pre-synthesis Cleaning

1.8.1. Ensure power is on to all peripheral devices.

1.8.2. Start the Profibus and Synthia controller on the Synthia PCI if necessary. Switch to screen II.

1.8.3. Start the program "Visual Chemistry" from screen II. Select 'Run Synthesis' after restart. Select the recipe "**CLEANING**" (refer to Appendix B).

1.8.4. Start the recipe.

1.8.5. The recipe will prime and flush all lines as well as clean the HPLC injection ports.

1.9. Dose vial

1.9.1. Remove the flip-top from a sterile empty vial. Weigh the vial using the verified portable balance. Record the weight on the Master Batch Record.

1.9.2. Prepare the laminar flow hood for operations.

1.9.2.1. Transfer the following materials to the laminar flow hood. Re-spray the interior and contents of the laminar flow hood with 70% isopropanol. Allow to dry.

Tared Sterile vial 10 mL; 1 each	4 mm Sterile Millex-GV filter
25 mm Sterile Millex-MP filter	Three 2", 21G sterile needles
1.5", 20 G sterile needle	Sterile Saline
10 mL sterile syringe	Sterile alcohol wipe

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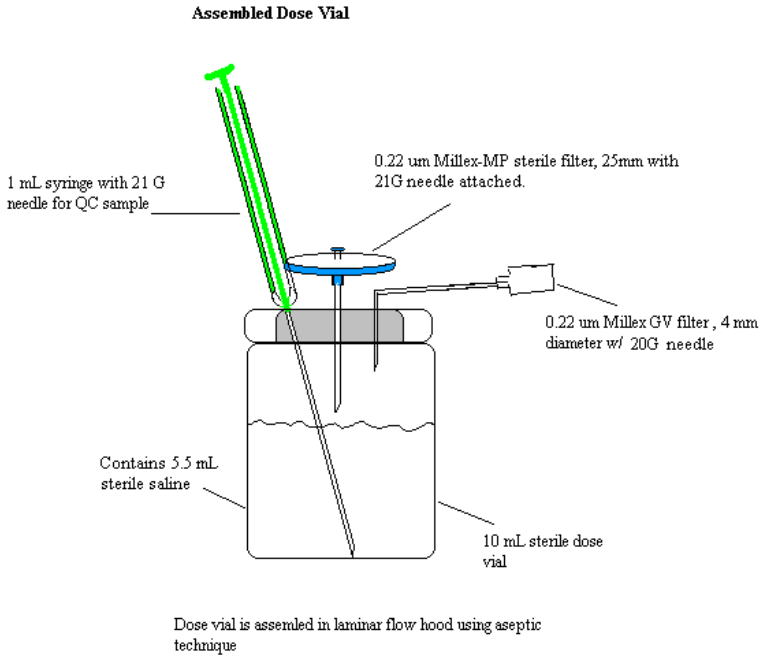
1.9.2.2. Assemble the dose vial as depicted in Figure 2 using aseptic technique. Wipe the top of the dose vial with an alcohol wipe and allow to dry prior to assembly.

Figure 2



1.9.3. Wipe the septa on the sterile saline with an alcohol wipe and allow to dry. Transfer 5.5 mL sterile saline to the dose vial using a sterile syringe.

1.9.4. Install the dose vial to the transfer line attached to B15 when ready for operations.

1.10. HPLC Setup

1.10.1. Preparative

1.10.1.1. Turn on the UV lamp of preparative HPLC system. Verify that the Beckman software is communicating with UV detector and pump, confirm that UV lamp reads ‘calibration done’.

1.10.1.2. Install the prep HPLC column and mobile phase solvents. Ensure that the column is leak free.

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1.10.1.3. Equilibrate the column for a minimum of 10 column volumes at initial conditions (refer to Table 2). The initial pressure for a new column should be no more than 3000 psi at 3 mL/min when fully equilibrated. Higher pressures indicate a possible clog in the column. The flow may be turned down once the column is equilibrated.

1.10.2. Analytical

1.10.2.1. Turn on the UV lamp of analytical HPLC system. Verify that the Beckman software is communicating with UV detector and pumps, confirm that UV lamp reads 'calibration done'.

1.10.2.2. Install the analytical HPLC column and mobile phase. Ensure that the column is leak free.

1.10.2.3. Equilibrate the column for a minimum of 10 column volumes at initial conditions. The initial pressure should be approximately 1700 psi at 1 mL/min once at full equilibration. The flow may be turned down once the column is equilibrated.

1.10.3. System Suitability for the analytical HPLC

1.10.3.1. Inject a system blank of 100 μL mobile phase to confirm a stable baseline.

1.10.3.2. Inject FBR standard (5-10 ng typical). Refer to SOP # QA304, Analytical HPLC Quality Control Method, for acceptance of the standard injection.

1.10.3.3. Inject 100 μL of formulated vehicle blank (nominally 10% dehydrated ethanol in sterile saline). Any small peaks observed in the formulated vehicle blank may be taken into account when analyzing the final product but a stable baseline should be observed.

1.10.3.4. Printouts of the system suitability data should be included in the batch record.

Table 2. HPLC Systems and Method Parameters

	Preparative	Analytical
Pump	Beckman 126	Beckman 126
Detector	Beckman 166	Beckman 166 or 168
Column	Luna C_{18} 3μm, 150 x 10 mm	Luna Phenyl Hexyl 5 μm, 150 x 4.6 mm
Flow Rate	3 mL/min	1.0 mL/min
Typical initial pressure	2800psi	1700psi
Gradient/ Isocratic	Gradient	Isocratic
Mobile Phase A	Water	10 mM AF 60% MeCN(aq)
Mobile Phase B	Acetonitrile	–
UV Wavelength	230 nm	230nm
Bioscan Setting	200k	20 M
Method Name	FBR Prep	FBR Analytical QC
Trigger	External	External
Sample Loop Size	5 mL	200 μL

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Date of review: Draft 8/17/2009

SOP # MP202

Production of *[^{18}F]FBR for Injection*. Part 2: Synthesis and Formulation

Approved by: _____ Initials;_____ Date:_____

Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: Synthesis and formulation of *[^{18}F]FBR for Injection*

1. Drying
   1.1. Program Setup
      1.1.1. Ensure power is on to all peripheral devices.
      1.1.2. Restart Visual Chemistry as required. Select 'Run Synthesis'. Select the recipe "**DRYING**" (refer to Appendix B).
      1.1.3. Start the recipe.

      Note: Prompts will appear through out the recipe requesting confirmation that a step has been completed before proceeding. The operator is instructed to visually confirm the step before pressing 'OK'.
      1.1.4. The recipe will ask "Ready to Start?" Visually confirm that all reagents are in position before pressing OK.
      1.1.5. The recipe will prompt the operator to load the $^{18}F/H_2{}^{18}O$/ Kryptofix solution.
   1.2. Azeotropic Drying
      1.2.1. Measure the activity of the vial containing the $^{18}F/H_2{}^{18}O$/ Kryptofix in the dose calibrator. Record the measurement and time on the Master Batch Record.
      1.2.2. Load the activity vial in position F33. Hit OK. The $^{18}F/H_2{}^{18}O$/ Kryptofix solution will be transferred in 250 μL aliquots to the thermal drying oven. Up to 750 μL of $^{18}F/H_2{}^{18}O$/ Kryptofix solution may be dried.
2. Synthesis
      2.1.1. Microwave Drying
         2.1.1.1. Once thermal drying is complete, close the Synthia program.
         2.1.1.2. Restart Visual Chemistry as required. Select 'Run Synthesis' after restart. Select the recipe "**SYNTHESIS**" (refer to Appendix B).

2.1.1.3. The activity in the thermal oven is reconstituted in anhydrous MeCN and transferred to the microwave oven via the Synthia. The activity is further dried via two cycles in the microwave. The Synthia recipe will prompt the operator to begin microwave drying. Microwave operations are performed manually by pressing the start button once the settings have been adjusted. Use the settings in section 2.1.1.4.1, Microwave Settings for Drying

2.1.1.4. The Synthia recipe will prompt the operator to begin microwave drying.

2.1.1.4.1. Microwave Settings for Drying

Power: 90 W
Temperature: 130 °C
Time: 150 s
Number of cycles = 2

Note: The preparative HPLC should be made ready for injection (HPLC method = FRB Prep.met) before proceeding as the Synthia recipe loads the injector loop and begins the HPLC run automatically. Ensure that the flow rate is at 3 mL/min. Check that the pressure is within the expected range.

2.1.2. Microwave Synthesis

2.1.2.1. Microwave operations are performed manually by pressing the start button once the settings have been adjusted. Use the settings in section 2.1.2.1.1; Microwave Settings for Synthesis. Press START to begin. Keep a careful eye on the temperature as the microwave synthesis proceeds. Should the temperature rise above 70 °C. Stop the run and allow it to cool to below 50 ° C then proceed. Keep track of the total time.

2.1.2.1.1. Microwave Settings for Synthesis

Power: 15 - 35W
Temperature: 70 °C
Time: 60 s
Number of Cycles = 2 **OR** Total Time = 120 s

2.1.3. After a brief cool-down, the Synthia recipe will dilute the reaction mixture with 700 μL water and inject onto the preparative HPLC.

2.1.4. A popup screen will appear asking if the operator wishes to collect into the large collection tube. Collect the product peak that elutes at approximately 78 minutes. Refer to Appendix D for a sample preparative trace.

2.1.5. The Synthia recipe will be finished. Stop the recipe and exit the program.

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2.1.5.1. The HPLC column should be cleaned with a minimum of 20 column volumes of 100% acetonitrile after absorbance is no longer observed in the trace.

3. Formulation

3.1.1. Restart Visual Chemistry. Select 'Run Synthesis' after restart. Select the recipe "**FORMULATION**" (refer to Appendix B).

3.1.2. Start the recipe.

3.1.3. The collected product is further diluted then passed through the SPE cartridge to concentrate it and remove acetonitrile. The product is then eluted from the SPE cartridge with the dehydrated ethanol for injection.

3.1.4. Visually verify that the product has been collected into the vessel at position E20.

3.1.5. The Gilson Aspec arm will pull up first sterile saline then the product in ethanol. The bolus is loaded through B15 through the sterile syringe filter to the sterile dose vial.

3.1.6. Complete the Master Batch Record.

3.1.7. Perform all required release and post release Quality Control Procedures according to the procedures outlined in SOP# QA302.

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Date of review: Draft 8/17/2009

SOP # QA301

Release of [^{18}F]*FBR for Injection*

Approved by: _____ Initials;_____ Date: _____

Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: **Description of release procedure and criteria for *[^{18}F]FBR for Injection.* Clarification of release and post-release test requirements.**

1. Release Tests

1.1. The following tests must be completed and all acceptance criteria met BEFORE release from the PRSS/MIB/NIMH production site. Refer to SOP# QA 302 for detailed procedures.

Test	Test description	Acceptance Criteria
pH	Narrow range pH paper	pH 4.5 to 8.0
Membrane filter integrity	Determines whether the membrane has remained intact	No bubbles observed at 45 psi
Appearance test	Visual inspection	Clear, colourkess, free of particulates
Chemical purity	HPLC	Amount of impurity not to exceed 1 μg for injected volume.
Cold Carrier Limit	HPLC	Amount of cold FBR not to exceed 10 μg for injected volume.
Radiochemical purity	HPLC	≤ 95%
Radiochemical identity	HPLC	t_r within 1 min of standard (corrected for UV to γ detector delay)
Radio-concentration	Measured activity divided by measured volume.	Not less than 0.5 mCi/ mL
Specific radioactivity[4]	Measured activity divided by carrier as quantified by HPLC	Not less than 500 mCi/ μmol at EOS
Radionuclidic Identity	Half life calculated from two different measurements at least 5 minutes apart.	Experimentally determined half life 110 ± minutes
Residual Solvent	Gas Chromatography	≤.4.1 mg acetonitrile in the injected dose[5]

[4] At End of Synthesis
[5] Refer to USP <467>

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		≤1 x 10^5 ng/ µL ethanol

2. Labeling and Release

2.1. If the product meets all acceptance criteria for the above tests, the product vial is labeled with the following information:

[18F]FBR for Injection

Caution: New drug limited by Federal law to investigational use only
4 h after calibration Half-life of 18F is 109 min
Concentration:_____mCi/mL Volume: :_____mL
Activity:_____mCi Time:_____
Calib. Date:_____ Lot #:_____

Duplicate labels are attached to the Post Release Test Record: Endotoxin and Sterility. Radioactivity is reported at the time of End Of Synthesis (EOS).

2.2. Sign and date the Quality Control Record authorizing release from the production/quality control area.

The dose vial containing the product may then be transported to the PET center.

3. Post Release Tests

3.1. The following tests must be completed within the time frame and with the frequency specified. Refer to SOP# 302 for detailed procedures.

Test	Test description	Acceptance Criteria
Bacterial endotoxins	*Limulus* Amebocyte Lysate (LAL)	None detected at 2.5 eu/ mL level
Sterility	Aerobic and anaerobic bacterial test	No (aerobic or anaerobic) growth observed

3.2. Quality control tests for sterility and bacterial endotoxins (LAL test) are to be performed on each batch of *[18F]FBR for Injection*. However, because of the time needed to perform these tests, the *[18F]FBR for Injection* may be released before test completion.

3.3. Bacterial endotoxin testing should be completed within 24 h of EOS of *[18F]FBR for Injection*.

3.4. A sample for sterility testing should be submitted to NIH Laboratory Medicine, Microbiology Department as soon as the radioactivity in the sample is below the detection level of a pancake detector; typically 48 hours. In the case where *[^{18}F]FBR for Injection* that was produced on a Friday or a workday preceding a Federal holiday, sampling and submission of the sterility test sample may be delayed until facilities are again open and personnel available.

3.5. Test results and reports should be included on the Post Release Test Record: Endotoxin and Sterility

4. Batch Record.

4.1. The batch record should include the following:

- Calculations Worksheet
- Master Batch Record
- Quality Control Record
- Post Release Test Record: Endotoxin and Sterility
- Preparative HPLC Report
- Analytical system suitability HPLC Reports (system blank, standard injection, vehicle blank)
- Analytical HPLC Report
- GC analysis report
- Sterility Report
- Any ancillary data obtained in the course of production of *[^{18}F]FBR for Injection*

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Date of review: Draft 8/17/2009

SOP # QA302

Sampling and Quality Control Procedures for *[^{18}F]FBR for Injection*

Approved by: _____ Initials;_____ Date:_____

Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: Sampling and Quality Control Procedures for *[^{18}F]FBR for Injection*

1. Sampling

1.1. The 1 mL sterile, sampling syringe previously inserted (under aseptic conditions) in the 10 mL dose vial containing *[^{18}F]FBR for Injection* is used to remove a 500 μL (minimum) sample.

1.2. Dispense 200 μL (minimum) to a clean vial for HPLC and other analyses.

1.3. Dispense 200 μL (minimum) to a pyrogen free test tube.

1.4. Use the samples as directed in this document.

2. Release Tests

2.1. pH

2.1.1. Dispense one drop of the QC sample onto the pH paper to measure pH. Record the pH on the Quality Control Record.

2.2. Appearance

2.2.1. Visually inspect the contents of the finished product. The product should be a clear, colorless liquid free of particulate or cloudiness.

2.3. Filter Integrity

2.3.1. Remove the intact filter assembly (filter and needle) from the sterile dose vial. The filter must be fully wetted for the test to be valid. If the operator suspects that the filter is not fully wetted, an additional 5 – 10 mL of saline may be passed through the filter before testing.

2.3.2. Obtain a small glass vessel containing water. Submerge the tip of the needle in the water.

2.3.3. With the air off, attach the filter to the compressed air supply in the hot cell. Turn the house air on. The initial pressure should be set to less than 10 psi.

2.3.4. Increase the pressure to 45 p.s.i. If no bubbles are observed at the needle outlet of the sterile filter when the pressure gauge reads 45 p.s.i., then the filter passes the test.

2.3.5. The result of the filter integrity test is recorded in the summary section of the QC results form.

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2.4. HPLC Analysis and Calculated Results

2.4.1. Measure the radioactivity of a 100 μL (typical) aliquot of *[^{18}F]FBR for Injection*

2.4.2. Analyse the sample according to the procedures found in SOP # 304, Analytical HPLC Quality Control Method.

2.4.3. Measure the residual radioactivity in the syringe after injection. Calculate the net radioactivity injected decay-corrected to EOS[6].

$$\text{mCi}_{(\text{Net})} = \text{radioactivity of full syringe} - \text{residual radioactivity} \quad \textbf{Eq. 1}$$

2.4.4. Integrate the UV trace. Integrate the BioScan trace.

2.4.5. Chemical Purity is determined from the UV trace by the equation:

$$\mu\text{g impurity} = \frac{\text{peak area impurity}}{\text{m}} \text{ x MW} \quad \textbf{Eq. 2}$$

Where *m* is the slope from the valid calibration curve in units of peak area *x* μmol^{-1}.

2.4.6. Radiochemical Purity is determined from the BioScan trace by the equation:

$$\%Purity = \frac{\text{Product Peak Area}}{\text{Total Peak Area}} x100 \quad \textbf{Eq. 3}$$

2.4.7. Radiochemical Concentration in units of mCi/ mL is determined from the net radioactivity in the 100 μL aliquot using the equation:

$$Concentration = \frac{\text{mCi}_{(\text{Net})}}{100\ \mu L} x \frac{1000\ \mu L}{1\ \text{m}L} \quad \textbf{Eq. 4}$$

2.4.8. Chemical and Radiochemical Identity

2.4.8.1. Compare the retention time of the product to that of the standard. The retention time of the product must be within 1.0 minute of the standard. The γ trace retention time must be within 1.0 minute of the standard corrected for any delay between the UV and γ detectors which are in series.

[6] End of Synthesis

2.4.9. Specific Activity is calculated from the amount of non-radioactive carrier in the injected aliquot and the net radioactivity in the same aliquot. The amount of carrier is determined from the valid calibration curve using the equation:

$$\mu\text{mol carrier} = \frac{\text{peak area carrier}}{\text{m}} \qquad \textbf{Eq. 5}$$

Where the slope is in units of area x μmol^{-1} and the area is from the UV trace at 230 nm. Specific Activity is calculated by:

$$s.a. = \frac{\text{mCi}_{(\text{Net})}}{\mu\text{mol Carrier}} \qquad \textbf{Eq. 6}$$

2.4.10. Radionuclidic Identity is determined from the experimentally determined half life calculated from two measurements taken at least 5 minutes apart.

$$t_{1/2} = \frac{\ln(2) \text{ x } \Delta t}{\ln\left(\frac{A_0}{A}\right)} \qquad \textbf{Eq. 7}$$

Where A_0 is the first radioactivity measurement, A is the second activity measurement, and **Δt** is the difference in time in units of minutes.

2.5. Residual Solvent

2.5.1. Verify that the instrument is ready for operations according to the procedures found in SOP # QA303.

2.5.2. Add 50 μL of *[^{18}F]FBR for Injection* (test sample) into a prepared auto sampler vial containing 50 μL of the calibrated propionitrile standard solution. Make sure that no air bubbles are present.

2.5.3. Load the prepared sample into position. Start the run. Data acquisition may be stopped and analysis performed at 6.0 minutes.

2.5.4. The sample is acceptable if the amount of acetonitrile (MeCN, t_R *ca.* 2.67 min) is less than 4.1 x 10^2 ng for the injected volume and the amount of ethanol (EtOH, t_R *ca.* 2.27 min) is less than 1 x 10^5 ng/ μL.

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3. Post Release Tests

The following tests are completed after release. Any test result that does not meet the passing criteria must be reported to the PRSS Chief.

3.1. Endotoxin Testing Procedures

Note: An alternative test method may be used provided that it is an FDA approved method and that documentation and department approval of the procedures used are included with the batch records. The FDA shall be notified should the substitution become permanent.

3.1.1. Label the appropriate tubes according to Table 2. found in the Post Release Test Record: Endotoxin and Sterility. Note that each test is run in duplicate.

3.1.2. Turn on the heat block and confirm temperature setting is 37 °C.

3.1.3. Wipe each vial stopper top with alcohol swabs. Label the Positive Control and Sample vials with a radioactivity sticker.

3.1.4. If is it necessary to make a 500 eu/ mL stock solution, add sterile WFI (USP) to a new CSE vial to produce a 500 eu/ mL stock solution. Mix well to ensure complete homogeneity. Label the vial as "500 eu/mL CSE" with the preparation date and expiry date (1 month). Store at 2 – 8 °C.

3.1.5. To produce the 5 eu/ mL CSE test solution, add 100 µL of the stock solution to a fresh 10 mL bottle of sterile WFI (USP). Label the vial as "5 eu/mL CSE" with the preparation date and expiry date (1 week). Store at 2 – 8 °C.

3.1.6. Dilute 200 µL of final formulated product with 800 µL of sterile water in a pyrogen-free test tube.

3.1.7. Add sample and control solutions to the tubes according to [18F]FBR For Injection: Post Release Test Record: Endotoxin and Sterility: Endotoxin and Sterility, Table 1.

3.1.8. Gently mix all contents of all vials and incubate at 37°C for 60 min.

3.1.9. After approximately one hour, check for the formation of gel in each tube by inverting the vial 180° in one smooth motion. If a gel has formed and remains intact in the tube bottom, the test result is considered to be positive. If no gel has formed or if the gel collapses the test result is negative.

3.2. Sterility

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3.2.1. The sterility sample is prepared from the remainder of the product in the original dose vial. Verify the absence of residual radioactivity using a GM detector.

3.2.2. Prepare a laminar flow hood for aseptic use.

3.2.3. Using a permanent marker, record lot # of decayed radiopharmaceutical and date of submission on the two blank lines of each of the Bactec vials. **Do not write on barcode/peelable barcode of the Bactec vials.**

3.2.4. Place the following in the hood:

Product dose vial
Bactec aerobic and anaerobic bottles
Appropriate sterile syringe
Aseptic alcohol wipe

3.2.5. Respray the hood with 70% isopropanol and allow to dry before proceeding.

3.2.6. Use aseptic technique to enter the laminar flow hood and prepare the samples.

3.2.7. Remove the flip-top caps of the aerobic and anaerobic Bactec vials.

3.2.8. Wipe the septum of the Bactec and product vial. Allow to dry.

3.2.9. Remove approximately 200 μL from the dose vial. Add approximately 100 μL each to the anaerobic and aerobic Bactec bottles.

3.2.10. Fill out the "Request for Sterility test" form and submit the sample and form to the NIH Clinical Center Microbiology Lab.

3.2.11. Attach the completed sterility test results to the Post Release Test Record: Endotoxin and Sterility.

3.2.12. *In Case of Positive Result*. If growth is reported: a) notify the PI; b) ask for the identity of the organism from the microbiology lab; c) file a report on the investigation and follow-up results in the GMP investigations file.

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Date of review: Draft 8/17/2009

SOP # QA303

Analysis of Organic Residues in *[18F]FBR for Injection* by Gas Chromatography

Approved by: _____ Initials;_____ Date:_____

Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: To test for volatile solvent residues in *[18F]FBR for Injection*

1. Operation
   1.1. Verify that the instrument is ready for operations.
   1.2. Verify that the water level in the H_2-90 hydrogen generator is sufficient.
   1.3. Verify that the H_2 pressure is at approximately 28 psi and that the He tank pressure is about 60 psi
   1.4. Verify that the vials in solvent positions A and B have sufficient DI water.
   1.5. Verify that FID is lit and the background signal is about 5.
   1.6. Load the method 'ISPRCN.M'
   1.7. Add equal amounts of *[18F]FBR for Injection* (test sample) and the calibrated propionitrile standard (50 mL of each is typical).into a prepared auto sampler with insert if required. Make sure that no air bubbles are present.
   1.8. Load the prepared sample into position. Start the run. The run may be stopped and analysis performed at 6.0 minutes.

      The sample is acceptable if the amount of acetonitrile (MeCN, t_R *ca.* 2.67 min) is less than 4×10^2 ng and the amount of ethanol (EtOH, t_R *ca.* 2.27 min) is less than 1×10^5 ng based on a 1 μL injection.

   1.9. Include a copy of the GC report in the Batch Record.
2. Post-run.
   2.1. Remove all samples and label radioactive samples. Download the method 'Default.M' that is used to maintain the oven temperature at 150 ˚C when GC is idle.

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Date of review: Draft 8/17/2009

Table 1. Materials and equipment

Agilent 6850 GC with flame ionization detector (FID)	Agilent 6850 series autosampler
J & W DBWAX column, 30 m (l) × 0.25 mm (id) × 0.25 μm (film thickness) (Alltech, part # 122-7032)	Acquisition and data processing software: GC Chem Station (version: Rev. A.09.03 [1417])
Inlet liner: split inlet glass liner with glass wool packing (Agilent part number, 5183-469119251-60540)	Parker Balston H2-90 Hydrogen Generator
High purity grade (99.995 %) compressed helium (Roberts Oxygen, cat. no. R 102 F3)	In-house air purified by Parker Balston Zero Air Generator, Model 75-83NA
In-house deionized water (18 MΩ) purified by Millipore Milli-Q;	Autosampler glass vial (Agilent part no. 5182-0864);
Internal Standard: 386 ppm propionitrile (aq).	Autosampler conical glass insert (Agilent part no. 5183-2085)

Table 2. Method (ISPRCN.M) parameters.

Injection port:			**Carrier gas:**
split sample injection			Helium 2 mL/min
split ratio of 20: 1 T = 250 ˚C			
Column temperature gradient:			**Detector:**
Time	Temperature	Duration	FID 250 °C
t_0	T=50 ° C	1 min	H_2 40 mL/min and air at 450 mL/min.
$t_{1\ min}$	T = 150 °C	5 min	He make-up 45 mL/min. Detector
$t_{6\ min}$	T = 150 °C	0.5 min	
$t_{6.5\ min}$	T = 220 °C	1.4 min	
$t_{7.9\ min}$	T = 220 °C	3 min	
$t_{10.9\ min}$	T = 50 °C	0 min	
Autosampler:			**Needle/Syringe wash:**
Syringe size		10 μL	Before injection 4
Sample injection volume		1 μL.	After injection 2

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SOP # QA304

Analytical HPLC Quality Control Method

Approved by: _____ Initials;_____ Date:_____

Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: To perform analytical HPLC QC on *[^{18}F]FBR for Injection*

1. Preparation

1.1. Prepare the HPLC system according to the parameters listed in Table1.

Table 1 Analytical System and Method Paramters

Component/ Parameter	
Pump	Beckman 126
Detector	Beckman 166 OR 168
Column	Luna Hexyl-Phenyl 3μm, 150 x 4.6mm
Guard/ Inline Filter	none
Flow Rate	1.0 mL/min
Typical pressure	1700 psi
Gradient/ Isocratic	Isocratic
Mobile Phase	10 mM AF 60% MeCN (aq)
UV Wavelength	230 nm
Bioscan Setting	20k
Trigger	Manual
Sample Loop Size	200 μL

1.2. Equilibrate the column for a minimum of 20 column volumes at initial conditions (refer to Table 1). The initial pressure should be approximately 1700 psi at 1.0 mL/min once at full equilibration. The flow may be turned down once the column is equilibrated.

1.3. Inject 100 μL (typical) of mobile phase as a system blank. If any anomalies in the baseline such as stray peaks or drift are present, the system blank should be repeated after the cause is identified and the problem corrected.

1.4. Inject an accurate volume of a known concentration of FBR standard (typically 5 to 10 ng) and analyse per SOP # QA304. The peak area must be within ± 10% of the expected peak area to be acceptable. In the event that the standard does not meet the criterion, a new calibration curve should be made.

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1.5. Record the area and retention time on the QC record.

2. Quantitative analysis of *FBR for Injection*

2.1. From the QC sample set aside according to the procedures found in SOP # QA302, remove 100 μL (typical) using a clean, dry HPLC syringe.

2.2. Measure the syringe contents in the dose calibrator. Record the measurement and the time on the *[^{18}F]FBR for Injection* Quality Control Record.

2.3. Inject the sample and re-measure the empty syringe. Record the measurement and the time on the *[^{18}F]FBR for Injection* Quality Control Record.

2.4. Decay correct the syringes (empty and full) to EOS and enter into the calculations worksheet.

2.5. When the HPLC run has finished, integrate the peaks. Record the retention time of the UV peak, the percent area of the UV peak, the percent area of the gamma peak on the QC form.

2.6. Record the peak area in the calculations worksheet.[7]

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[7] A copy of the calculations worksheet may be found in Appendix C

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SOP # QA305

FBR Precursor and Reference FBR Acceptance Criteria

Approved by: _____ Initials;_____ Date:_____

Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: **To establish the testing and acceptance criteria for FBR reference and precursor compounds.**

1. Overview

The procedure at PRSS/ MIB/ NIMH for the radiosynthesis of [^{18}F]FBR requires *N-(2,5-dimethoxybenzyl)-2-bromo-N-(2-phenoxyphenyl)acetamide* (hereafter referred to as Br-FBR precursor).

Reference FBR, *N-(2,5-dimethoxybenzyl)-2-fluoro-N-(2-phenoxyphenyl)acetamide* is required for quality control procedures. This non-radioactive material is used as a quantitative and qualitative standard for the determination of specific radioactivity and chemical identity.

Acceptance testing is required to verify the chemical purity and identity of both precursor and reference standard. Upon acceptance, the precursor and reference materials may be released for the production and quality control of *[^{18}F]FBR for Injection*.

This SOP contains the test methods and acceptance criteria for both compounds.
The set of acceptance tests include MS and NMR to establish chemical identity and LC-MS/ HPLC to establish chemical purity.

After acceptance, each lot of precursor and standard will be re-qualified annually for chemical purity using HPLC analysis and chemical identity using LC-MS analysis.

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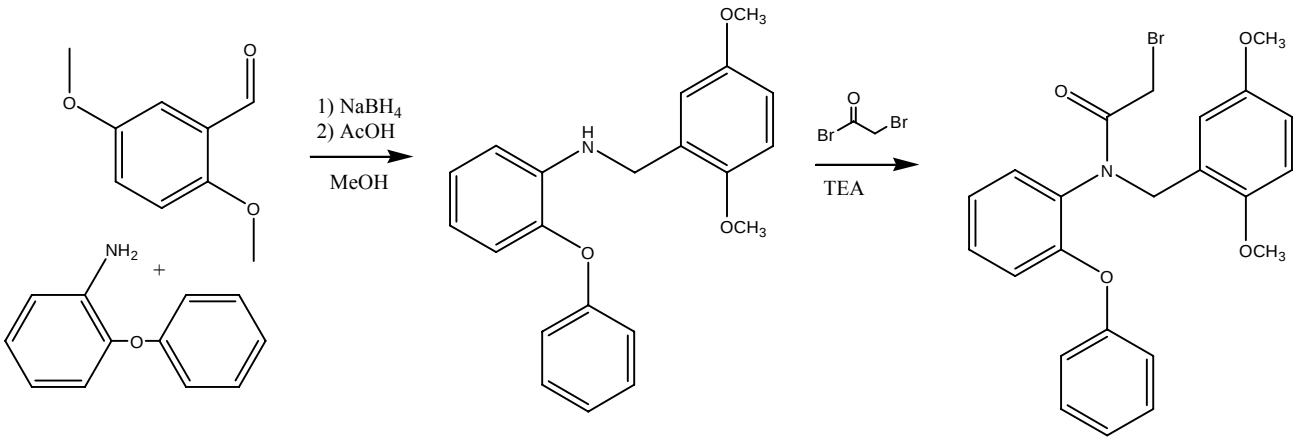
Date of review: Draft 8/17/2009

2. Synthetic Schemes for Preparation of Precursor and Reference Standards

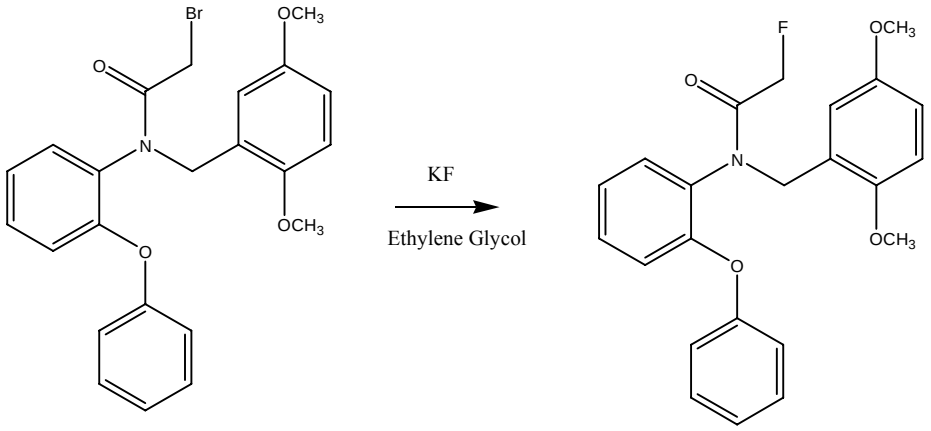
2.1. The precursor compound**,** *N-(2,5-dimethoxybenzyl)-2-bromo-N-(2-phenoxyphenyl)acetamide,* is synthesized from commercially available 2,5-dimethoxybenzaldehyde and 2-phenoxyaniline in two steps, according to Scheme A.

Scheme A. Br-FBR Precursor



2.2. The non-radioactive FBR reference compound is prepared by displacement of the bromo substituent by fluoride ion, as seen in Scheme B.

Scheme B. FBR Reference Compound



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3. Acceptance Test Method Parameters/ Sample Preparation

3.1. Material Source

The FBR precursor and reference FBR are currently produced by PRSS/ MIB/ NIMH but may be obtained from an outside source provided that all acceptance criteria contained in this document are met.

3.2. Acceptance Tests[8]

3.2.1.NMR

Sample Prep

Fifteen to twenty milligrams of the material to be tested (either FBR precursor or FBR reference material in $CDCl_3$.)

Instrument

Bruker Avance 400 NMR with TopSpin, ver. 1.3 software.

Methods

^{1}H-NMR	Method Name: Proton
^{13}C-NMR	Method Name: C13 CPD

3.2.2.LC-MS

Sample Prep

20 μg/mL FBR precursor in MeCN or MeOH

50 μg/ mL FBR reference material in MeOH.

Instrument

Thermo-Finnigan LCQ Deca LC/MS with Xcalibur V 2.0 software.

Method Parameters

Column:	Luna 5μm, 150 x 2 mm
Flow Rate:	150 μL/ min
Gradient/ Isocratic	Isocratic
Mobile Phase:	Methanol: Water: Acetic Acid 77.25: 22.25 : 0.5 %v/v
Injection Volume:	1 μL
Ionization:	Electrospray
Detection Range:	*m/z* 150 to 750

[8] Equivalent instrumentation and parameters may be used provided that it has been demonstrated that consistent results may be obtained..

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3.2.3. HPLC

Sample Prep
10 μg/mL FBR precursor or FBR reference material in 50% MeCN (aq).

Instrument
Beckman Coulter 126 pump and 166 or 168 detector with 32 Karat, ver. 7 software. Phenomenex Luna Hexyl-Phenyl 3 μm, 150 x 4.6 mm column.

Method Parameters
Refer to the method parameters found in SOP # 304, Analytical HPLC Quality Control Method

3.3. Acceptance Criteria

^{1}H-NMR and ^{13}C-NMR
Consistent with the structure. Refer to reference spectra attached

LC-MS
Molecular ion consistent with structure. Refer to reference spectra attached.

HPLC
Purity at 230 nm greater than 95%. Refer to reference spectra attached.

4. Documentation

4.1. The Precursor and Reference Acceptance Form should be filled out completely with the appropriate spectra attached. The completed form and attachments should be stored in the *[18F]FBR for Injection* Supplementary Records Binder and stored with *[18F]FBR for Injection* Batch Records.

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Date of review: Draft 8/17/2009

SOP # QA306

Standard HPLC Calibration Curve of Reference FBR

Approved by: _____ Initials;_____ Date:_____

Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: **To make a standard solution of FBR and generate a calibration curve to determine the mass of the carrier in *[18F]FBR for Injection***

Procedure

1. 0.1 μg/μL Stock solution of Reference FBR
   1.1. Remove the septum and seal from a 30 mL sterile vial. Re-crimp the butyl stopper in place with a new open ring seal such that the septum may be pierced with a beveled needle.
   1.2. Tare the vial. Record the weight.
   1.3. Dissolve 2.5 mg (nominal weight) FBR in a small amount of HPLC grade acetonitrile. Transfer the FBR solution to the tared vessel via a sterile syringe and needle through the septum.
   1.4. Vent the vial through the septum and place under inert gas (nitrogen or argon) flow to remove the solvent. When no solvent is visible, place the vented vial under high vacuum for at least 12 hours.
   1.5. Remove the vent needle and reweigh the vial. Record the weight and calculate the mass of FBR contained in the vial.
   1.6. Add anhydrous acetonitrile to the vial such that a 0.1 μg/μL (nominal) solution is made. Record the volume of acetonitrile (d = 0.782 g/ mL) added and calculate the concentration of the solution.
   1.7. Record the vial contents, concentration, storage conditions, and date made on the label. Store at $^{-}$20°C.
   1.8. The data and calculations are recorded on the FBR Quantitative Standard Form. The form is filed in the *[18F]FBR for Injection* Supplementary Records Binder and stored with *[18F]FBR for Injection* Batch Records.
2. 0.1 ng/ μL (4 x 10^{-2} μmol/ mL) Standard Solution
   2.1. Remove the FBR Stock solution from storage and allow to warm to room temperature.
   2.2. Remove a sufficient amount of the stock solution (typical 150 μL) of the solution to make the required standard solution and place in a clean auto-sampler vial with cap.

2.3. Dilute 1:1000 with 50% v/v acetonitrile (aq). (example: 50 µL in 100 mL acetonitrile solution using a syringe to measure the volume of stock). Mix well.

2.4. Transfer the standard solution to a sterile 30 mL vial with septum and crimp seal.

2.5. Record the vial contents, concentration, storage conditions, and date made on the label. Store at ⁻20°C.

2.6. The data and calculations are recorded on the FBR Quantitative Standard Form. The form is filed in the *[^{18}F]FBR for Injection* Supplementary Records Binder and stored with *[^{18}F]FBR for Injection* Batch Records.

3. Calibration Curve

3.1. Remove the FBR standard solution from storage and allow to warm to room temperature.

3.2. Remove a 1 mL aliquot (approximately) of the solution and place in a clean auto-sampler vial with cap.

3.3. Perform replicate (minimum five) injections of a minimum of 4 different volumes (the range in volume should be chosen such it brackets the span of the expected concentration range of *[^{18}F]FBR for Injection*; approximately 1 x 10^{-5} to 6 x 10^{-5} µmol) of the standard solution should be analysed using the instrument and method parameters found in SOP # QA304: Analytical HPLC Quality Control Method.

3.4. Calculate the molar mass of each injection volume. (The molecular weight of FBR is 395.4 g/mol)

3.5. Calculate the mean and percent relative standard deviation (%RSD) of each replicate set. The %RSD must be ≤ 3%. In the event that the %RSD of a replicate set is greater than 3%, the replicate set must be repeated unless a single injection may be excluded for cause.

3.6. Calculate the linear fit of the mean peak area as a function of the µmoles injected. Include the point (0,0) (forced origin). Report the slope in units of **area** x **µmol** $^{-1}$. Plot the data.

3.7. Calculate the correlation coefficient (r^2). r^2 must be ≥ 0.98.

3.8. The data and calculations are recorded on the FBR Calibration Curve Form. The form, together with copies of all HPLC chromatograms and printout of the calibration curve, is filed in the *[^{18}F]FBR for Injection* Supplementary Records Binder and stored with *[^{18}F]FBR for Injection* Batch Records.

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Appendix A

Materials, Instruments, and Equipment

This appendix lists the materials and equipment used in the production and quality control procedures for *[^{18}F]FBR for Injection*.

Materials:

Materials	Manufacturer or Supplier	catalog #
0.22 μm Sterile 25 mm Syringe Driven Filter Unit; Millex MP	Millipore	SLMP L25 SS
0.22 μm Sterile 4 mm vent filter; Millex GV	Millipore	SLGV0004SL
0.45 micron nylon membrane filter	Phenomenex	AF0-0504
0.5 mL sterile insulin syringe	Becton-Dickinson	329465
1 mL tuberculin syringe	Henke Sass Wolf	CE0123
LAL test materials	Cape Cod	Ref. Document 5
10 mL sodium chloride, USP for injection	American Pharmaceutical Partners, Inc.	NDC 63323-186-10
10 mL Sterile serum vial	Abbott Laboratories	5816-11
30 mL Sterile serum vial	Abbott Laboratories	5829-30
Acetonitrile, Reidel de Haën DNA Synthese grade	Sigma-Aldrich	34442
Acetonitrile HPLC grade	Burdick and Jackson	017-4
Alcohol Prep	Kendall	6818
Analytical column; C_{18} Phenyl-Hexyl, 3 μm, 4.6 mm × 250 mm	Phenomenex	00F-4256-E0
Ammonium Formate	Sigma-Aldrich	516961
Ethanol, USP	Warner-Graham	64-17-5
Nitrogen, Ultra High Purity Carrier Grade	Roberts Oxygen	R104A3
FBR	PRSS/ MIB/ NIMH	NA
FBR precursor	PRSS/ MIB/ NIMH	NA
Semi prep column; C_{18} Luna 10 micron, 10 mm × 250 mm	Phenomenex	00G-4094-N0
Sterile 20 G 1.5” needle	Becton-Dickinson	W12552
Sterile 21 G 2” needle	Becton-Dickinson	W12562
Water, HPLC Grade	EM Science	EMWX0004-1

Materials, equipment and reagents may be substituted with equivalent materials, equipment and reagents. All substitutions must be documented and approved by the PRSS chief.

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Instruments and equipment:

Operation/Function	Manufacturer	Model	Serial #
Radiosynthesis	General Electric (Formerly Uppsala)	Beatrice	002
HPLC purification	Beckman Coulter	System Gold 126 pump and 166 detector	3422360
HPLC Quality Control	Beckman Coulter	System Gold 126 pump and 168 detector	0412115
Radioactivity detection in HPLC purification	Bioscan	Flow-Count PIN detector	0605-315
Radioactivity measurement	Biodex	AtomLab300 dose calibrator	01332706
Mass measurement/volume of [^{18}F]*FBR for Injection*	Acculab	PP-250B balance	492AN025
Mass measurement of FBR precursor	Sartorius	CP225D analytical balance	13907271
Pressure regulator to test filter integrity	Porter Instrument Co.	40000AMVS60	NA
Verification of analytical and portable balances	ICL calibration labs	ASTM Class 1, 1.0 and 10.0 mg standard weights	2817
Measurement of HPLC sample for SA and carrier mass analysis	Hamilton	Microliter syringe, 100 μL , 84886	NA

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Date of review: Draft 8/17/2009

Appendix B

Synthia Methods and Positions

The positions at the time of this validation are shown in Table 1 of this appendix. The four recipes used to produce *[^{18}F]FBR for Injection* are found in Tables 2 through 4 of this appendix.
The positions in Synthia refer to the position of the arm and not the positions of the vials themselves. For this reason, it is important to have the correct size and shape vial in the assigned place.
Because the slight drift may occur, the positions should be periodically check and readjusted as necessary.

POSITIONS

Pos_name	X-pos	Y-pos	Z-pos		Pos_name	X-pos	Y-pos	Z-pos		Pos_name	X-pos	Y-pos	Z-pos
A1	0	0	570		E21	2791	1093	304		F30	2252	1962	1580
A2	76	0	436		E22	2791	1320	304		F31	1761	2192	615
A3	140	0	600		E23	2547	859	282		F32	1994	2219	602
B10	2230	114	390		E24	2547	1094	282		F33	2257	2178	397
B11	2478	115	564		E25	2547	1321	282		F34	1748	2405	72
B12	2708	114	385		E26	3044	365	296		F3	2258	397	625
B13	2958	114	385		E27	3044	589	296		F4	1758	643	617
B14	3208	102	371		E28	2791	365	304		F5	1994	627	419
B15	3458	113	551		E29	2803	600	258		F6	2234	630	640
B16	3871	111	553		E2	3050	855	1410		F7	1755	889	610
B1	266	117	387		E30	2547	365	282		F8	2013	888	623
B2	269	117	392		E31	2547	591	282		F9	2261	888	616
B3	510	118	401		E3	3057	1093	1410		H1	384	672	989
B4	767	117	387		E4	3062	1322	1404		H2	372	2494	562
B5	1006	116	389		E5	2817	899	1318		I10	2792	1457	275
B6	1242	116	397		E6	2798	1097	1412		I1	2792	1661	300
B7	1492	116	390		E7	2798	1314	1407		I2	2792	2400	300
B8	1739	116	389		E8	2554	876	1407		I3	2792	2424	300
B9	1975	114	389		E9	2562	1097	1418		I4	2792	1965	300
C1	3891	1382	565		F10	1738	1176	516		I5	2792	1706	300
C2	3891	1156	573		F11	1984	1176	513		I6	2792	745	275
C3	3891	932	602		F12	2242	1180	514		I7	2802	727	300
C4	3891	706	605		F13	1704	1432	329		I8	2809	1007	304
C5	3891	464	647		F14	1864	1437	348		I9	2792	1230	275
D1	3462	426	454		F15	2000	1432	356		J10	3518	356	0
D2	3440	717	448		F16	2157	1432	329		J1	2470	325	0
D3	3448	1018	445		F17	2301	1432	330		J2	2760	329	0
D4	3463	1319	287		F18	1686	1579	373		J3	2965	325	0
D5	3275	2234	240		F19	1840	1579	382		J4	2470	570	0
D6	3468	1638	618		F1	1740	381	640		J5	2714	570	0
E10	2560	1327	1415		F20	1989	1579	379		J6	2960	570	0

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

E11	3047	2263	734		F21	2138	1579	384		F99	2245	1938	1586
E12	2803	2280	731		F22	2292	1577	363		J99	3034	1920	0000
E13	2572	2282	736		F23	1695	1727	804		H3	1187	2301	0713
E14	3046	2263	527		F24	1840	1732	810		J13	3299	2211	0
E15	2791	2268	527		F25	1984	1725	801		J93	1908	2252	0
E16	2550	2268	527		F26	2132	1727	802		H4	374	2186	426
E17	3044	857	296		F27	2282	1725	804		H5	374	2186	217
E18	3044	1092	296		F28	1766	1949	423		H6	321	2097	047
E19	3044	1319	296		F29	1987	1956	386		B17	1298	1611	212
E20	2808	878	255		F2	1992	373	621					

RECIPES

CLEANING

Row_nr	Object	Function	Arg_1	Arg_2	Arg_3	Arg_4	Label	Comments
1	Dout	Reset_All	#	#	#	#	#	Set all Dout devices to Off
10	#	Delay	5	#	#	#	#	Wait 3 s
20	Robot	Home	#	#	#	#	#	Position home
30	#	Delay	3	#	#	#	#	Wait 3 s
90	#	Print	Initiating cleaning procedure	#	#	#	#	
100	Robot	Move_XYZ	A1	#	#	#	#	Position rinsing needle
300	Dilutor	Init	L	N	#	#	#	Initiating the dilutor
600	Oven	Set_Temperature	8	18	#	#	#	Set evaporator temperature
700	Oven	Set_Temperature	7	100	#	#	#	Set evaporator temperature
800	Dout	Off	34	#	#	#	#	Sterile needles go up
900	#	Delay	1	#	#	#	#	Wait 2 s
1000	Dout	Off	36	#	#	#	#	Closing the trapdoor
1100	Dout	Reset_All	#	#	#	#	#	Set all Dout devices to Off
1200	Dout	On	35	#	#	#	#	Sterile vial slider out from sterile station
1300	Flow	Set_Flow	1	20	200	#	#	Setting nitrogen flow to 20 ml/min
1400	BCD	SET_Position	1	1	#	#	#	
1500	BCD	SET_Position	3	1	#	#	#	
2010	#	Attention	Check that all reagents are in position!	#	#	#	#	Wait 2 s
2030	#	Ask	Would you like to prime the dilutor?	No_Prime	#	#	#	
2040	#	Print	Priming the dilutor.	#	#	#	#	
2050	Dilutor	Init	L	N	#	#	#	Initiating the dilutor

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: Draft 8/17/2009

2100	Dilutor	Aspirate	L	5000	50	R	#	Priming the dilutor / Rinse needle
2200	Dilutor	Dispense	L	5000	20	N	#	Priming the dilutor / Rinse needle
2300	Robot	Move_XYZ	A1	#	#	#	#	Position rinsing needle
2400	Dilutor	Aspirate	L	10000	50	R	#	Priming the dilutor / Rinse needle
2500	Dilutor	Dispense	L	10000	20	N	#	Priming the dilutor / Rinse needle
2600	Dilutor	Aspirate	L	2000	50	R	No_Prime	Priming the dilutor / Rinse needle
2700	Dilutor	Dispense	L	2000	20	N	#	Priming the dilutor / Rinse needle
2800	Robot	Move_XYZ	A2	#	#	#	#	Position rinsing the top of the needle
2900	Dilutor	Aspirate	L	1000	50	R	#	Priming the dilutor / Rinse needle
3000	Dilutor	Dispense	L	1000	10	N	#	Priming the dilutor / Rinse needle
3100	#	Print	Rinsing the preparative injection port.	#	#	#	#	
3125	Robot	Move_XYZ	F3	#	#	#	#	Position rinsing preparative injection port
3130	Dilutor	Aspirate	L	3500	20	N	#	Rinsing the preparative injection port
3200	Robot	Move_XYZ	B11	#	#	#	#	Position rinsing preparative injection port
3250	Dilutor	Dispense	L	3500	10	N	#	Rinsing the preparative injection port
3300	Dilutor	Aspirate	L	5000	20	R	#	Rinsing the preparative injection port
3400	Dilutor	Dispense	L	5000	10	N	#	Rinsing the preparative injection port
3500	#	Print	Rinsing the analytical injection port	#	#	#	#	
3510	Robot	Move_XYZ	F3	#	#	#	#	Position rinsing preparative injection port
3520	Dilutor	Aspirate	L	3500	20	N	#	Rinsing the preparative injection port
3530	Robot	Move_XYZ	B16	#	#	#	#	Position rinsing preparative injection port
3540	Dilutor	Dispense	L	3500	10	N	#	Rinsing the preparative injection port
3550	Dilutor	Aspirate	L	5000	20	R	#	Rinsing the preparative injection port
3560	Dilutor	Dispense	L	5000	10	N	#	Rinsing the preparative injection port
3600	Robot	Home	#	#	#	#	#	

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: Draft 8/17/2009

12600	#	Print	Rinsing the needle.	#	#	#	No_Evap	
12700	Robot	Move_XYZ0	A1	#	#	#	#	Position rinsing needle
12750	Dilutor	Init	L	N	#	#	#	Initiating the dilutor
12800	Dilutor	Aspirate	L	4000	40	R	#	Rinsing the needle
12900	Dilutor	Dispense	L	4000	20	N	#	Rinsing the needle
13000	Robot	Move_XYZ	A2	#	#	#	#	Position rinsing the top of the needle
13100	Dilutor	Aspirate	L	3000	40	R	#	Rinsing the needle
13200	Dilutor	Dispense	L	3000	20	N	#	Rinsing the needle
13300	Dout	Off	33	#	#	#	#	Trap needles go up
14000	Flow	Set_Flow	1	200	200	#	#	Setting nitrogen flow to 20 ml/min
15000	Dout	On	30	#	#	#	#	Signalling to start analytical HPLC system
15850	Robot	Move_XYZ0	F8	#	#	#	#	
15880	Dilutor	Aspirate	L	1000	5	N	#	pulling up air plug
15900	Robot	Move_XYZ	F8	#	#	#	#	rinsing dilutor line w/MeCN
16000	Dilutor	Aspirate	L	3000	20	N	#	
16100	Robot	Move_XYZ0	A1	#	#	#	#	Position rinsing the needle on the outside
16200	Dilutor	Dispense	L	3000	20	N	#	purging dilutor line w/MeCN
16300	Robot	Move_XYZ	F8	#	#	#	#	
16400	Dilutor	Aspirate	L	2000	20	N	#	
16500	Robot	Move_XYZ0	A2	#	#	#	#	
16600	Dilutor	Dispense	L	2000	20	N	#	Rinsing the needle
16650	Robot	Move_XYZ0	A1	#	#	#	#	Needle in fresh MeCN vial
16675	Dilutor	Aspirate	L	1000	5	N	#	Pull up MeCN rinse of fluoride vessel
16678	Robot	Home	#	#	#	#	#	Position home
16680	Dout	Reset_All	#	#	#	#	#	#

DRYING

Row_nr	Object	Function	Arg_1	Arg_2	Arg_3	Arg_4	Comments
1	Dout	Reset_All	#	#	#	#	Set all Dout devices to Off
5	#	Delay	5	#	#	#	Wait 3 s
10	Robot	Home	#	#	#	#	Position home
15	#	Delay	3	#	#	#	Wait 3 s
20	#	Print	Initiating drying of fluoride	#	#	#	
25	Flow	Set_Flow	1	5	20	#	Setting nitrogen flow to 20 ml/min

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: Draft 8/17/2009

30	Oven	Set_Temperature	8	18	#	#	Set evaporator temperature
35	Oven	Set_Temperature	7	100	#	#	Set evaporator temperature
40	Dout	Off	33	#	#	#	Trap needles go up
65	BCD	SET_Position	1	1	#	#	
70	BCD	SET_Position	3	1	#	#	
75	Robot	Move_XYZ0	A1	#	#	#	Needle in fresh MeCN vial
85	Dout	On	29	#	#	#	Trap needles go up
90	Dout	On	43	#	#	#	Trap needles go up
95	Flow	Set_Flow	1	200	200	#	Setting nitrogen flow to 20 ml/min
100	#	Delay	30	#	#	#	Trap needles go up
105	Dout	Off	29	#	#	#	Trap needles go up
110	Dout	Off	43	#	#	#	Trap needles go up
115	Flow	Set_Flow	1	0	200	#	Setting nitrogen flow to 200 ml/min
125	Robot	Move_XYZ0	A1	#	#	#	Position rinsing needle
126	Flow	Set_Flow	1	200	200	#	Setting nitrogen flow to 20 ml/min
128	Dout	On	30	#	#	#	Signalling to start analytical HPLC system
130	Dilutor	Init	L	N	#	#	Initiating the dilutor
140	#	Attention	Ready for drying process?	#	#	#	Wait 2 s
150	#	Print	Load Fluoride in H1 and Clamp Vial	#	#	#	Fluoride vial added to Synthia rack
160	Dout	On	40	#	#	#	
165	#	Delay	2	#	#	#	
170	Dout	On	26	#	#	#	
180	#	Delay	2	#	#	#	
190	Dout	Off	40	#	#	#	
200	#	Print	Drying First volume fluoride	#	#	#	
300	#	Delay	2	#	#	#	
400	Robot	Move_XYZ0	F7	#	#	#	Rinsing original fluoride veesel again
420	Dilutor	Aspirate	L	800	5	N	Pull up 0.5 mL MeCN fr fluoride vessel
450	Robot	Move_XYZ	F7	#	#	#	Rinsing original fluoride veesel again
500	Dilutor	Aspirate	L	500	5	N	Pull up 0.5 mL MeCN fr fluoride vessel

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

600	Robot	Move_XYZ0	F33	#	#	#	Needle in fresh MeCN vial
700	Dilutor	Aspirate	L	100	5	N	Pull up MeCN rinse of fluoride vessel
800	Robot	Move_XYZ	F33	#	#	#	Needle in fresh MeCN vial
900	Dilutor	Aspirate	L	250	5	N	Pull up MeCN rinse of fluoride vessel
1000	Robot	Move_XYZ	H1	#	#	#	Needle in azeotrope vessel
1010	Dilutor	Dispense	L	900	5	N	Adding 3rd MeCN rinse
1020	Robot	Move_XYZ0	F7	#	#	#	Needle in fresh MeCN vial
1030	#	Delay	2	#	#	#	
1040	Robot	Move_XYZ	F7	#	#	#	Rinsing original fluoride veesel again
1055	Dilutor	Aspirate	L	600	5	N	Pull up 0.5 mL MeCN fr fluoride vessel
1060	Robot	Move_XYZ0	F33	#	#	#	Needle in fresh MeCN vial
1070	Dilutor	Aspirate	L	100	5	N	Pull up MeCN rinse of fluoride vessel
1080	#	Delay	200	#	#	#	
1095	#	Print	Drying Second volume fluoride	#	#	#	
1100	Robot	Move_XYZ	F33	#	#	#	Needle in fresh MeCN vial
1105	Dilutor	Aspirate	L	250	5	N	Pull up MeCN rinse of fluoride vessel
1110	Robot	Move_XYZ	H1	#	#	#	Needle in azeotrope vessel
1120	Dilutor	Dispense	L	900	5	N	Adding 3rd MeCN rinse
1130	Robot	Move_XYZ0	F7	#	#	#	Needle in fresh MeCN vial
1135	#	Delay	2	#	#	#	
1140	Robot	Move_XYZ	F7	#	#	#	Rinsing original fluoride veesel again
1150	Dilutor	Aspirate	L	600	5	N	Pull up 0.5 mL MeCN fr fluoride vessel
1160	Robot	Move_XYZ0	F33	#	#	#	Needle in fresh MeCN vial
1175	Dilutor	Aspirate	L	100	5	N	Pull up MeCN rinse of fluoride vessel
1180	#	Delay	200	#	#	#	

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: Draft 8/17/2009

1190	#	Print	Drying Third volume fluoride	#	#	#	
1200	Robot	Move_XYZ	F33	#	#	#	Needle in fresh MeCN vial
1210	Dilutor	Aspirate	L	350	5	N	Pull up MeCN rinse of fluoride vessel
1220	Robot	Move_XYZ	H1	#	#	#	Needle in azeotrope vessel
1230	Dilutor	Dispense	L	1000	5	N	Adding 3rd MeCN rinse
1240	Robot	Move_XYZ0	F7	#	#	#	Needle in fresh MeCN vial
1250	Robot	Move_XYZ	F7	#	#	#	Rinsing original fluoride veesel again
1260	Dilutor	Aspirate	L	550	5	N	Pull up 0.5 mL MeCN fr fluoride vessel
1265	Robot	Move_XYZ0	F7	#	#	#	Needle in fresh MeCN vial
1270	#	Delay	200	#	#	#	
1275	#	Print	Last Thermal drying of fluoride	#	#	#	
1280	Robot	Move_XYZ	H1	#	#	#	Needle in azeotrope vessel
1285	Dilutor	Dispense	L	600	5	N	Adding 3rd MeCN rinse
1287	Robot	Move_XYZ0	A1	#	#	#	Needle in fresh MeCN vial
1290	Dilutor	Init	L	N	#	#	Initiating the dilutor
1295	Dilutor	Aspirate	L	1100	5	N	Pull up MeCN rinse of fluoride vessel
1298	Dout	On	29	#	#	#	Trap needles go up
1300	Dout	On	43	#	#	#	Trap needles go up
1305	Flow	Set_Flow	1	200	200	#	Setting nitrogen flow to 20 ml/min
1310	#	Delay	30	#	#	#	Trap needles go up
1315	Dout	Off	29	#	#	#	Trap needles go up
1320	Dout	Off	43	#	#	#	Trap needles go up
1330	Flow	Set_Flow	1	200	0	#	Setting nitrogen flow to 20 ml/min
1350	Robot	Move_XYZ	F7	#	#	#	Needle in fresh MeCN vial
1355	Dilutor	Aspirate	L	1000	5	N	Pull up 0.5 mL MeCN fr fluoride vessel
1360	Robot	Move_XYZ0	F1	#	#	#	Needle in azeotrope vessel
1365	Dilutor	Dispense	L	1000	5	N	Adding 3rd MeCN rinse

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: Draft 8/17/2009

1370	Robot	Move_XYZ	F7	#	#	#	Needle in fresh MeCN vial
1375	Dilutor	Aspirate	L	1000	5	N	Pull up 0.5 mL MeCN fr fluoride vessel
1380	Robot	Move_XYZ0	F1	#	#	#	Needle in azeotrope vessel
1385	Dilutor	Dispense	L	1000	5	N	Adding 3rd MeCN rinse
1400	Robot	Move_XYZ0	A1	#	#	#	Needle in fresh MeCN vial
1410	Flow	Set_Flow	1	50	200	#	Setting nitrogen flow to 20 ml/min
1420	Dout	On	29	#	#	#	Trap needles go up
1430	Dout	On	43	#	#	#	Trap needles go up
1440	Flow	Set_Flow	1	200	200	#	Setting nitrogen flow to 20 ml/min
1450	#	Delay	120	#	#	#	Trap needles go up
1460	Dout	Off	29	#	#	#	Trap needles go up
1470	Dout	Off	43	#	#	#	Trap needles go up
1475	Flow	Set_Flow	1	0	200	#	Setting nitrogen flow to 200 ml/min
1480	Dilutor	Aspirate	L	500	5	N	Pull up MeCN rinse of fluoride vessel
1485	#	Delay	100	#	#	#	
1490	#	Attention	Drying is finished : perform Synthesis	#	#	#	
1500	Oven	Set_Temperature	6	18	#	#	Turn off reaction oven
1510	Oven	Set_Temperature	8	18	#	#	Turn off evaporator oven
1520	Robot	Home	#	#	#	#	Position home
1550	Vacuum	Stop	#	#	#	#	Turn off vacuum pump

SYNTHESIS

Row_nr	Object	Function	Arg_1	Arg_2	Arg_3	Arg_4	Comments
1	#	Delay	2	#	#	#	Wait 3 s
10	Robot	Home	#	#	#	#	Position home
15	#	Delay	3	#	#	#	Wait 3 s
20	#	Print	Initiating Synthesis	#	#	#	
50	Flow	Set_Flow	1	5	20	#	Setting nitrogen flow to 20 ml/min
100	Oven	Set_Temperature	7	100	#	#	Set evaporator temperature
500	#	Print	Transfering_Activity 1	#	#	#	
520	Robot	Move_XYZ0	F16	#	#	#	Needle in Rxn vessel

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: Draft 8/17/2009

540	Dilutor	Aspirate	L	1000	5	N	Adding resolubilized/dry fluoride
560	Robot	Move_XYZ	F16	#	#	#	Needle in Rxn vessel
580	Dilutor	Aspirate	L	700	5	N	Pull up 0.3 ml resolubilizing solvent
600	Robot	Move_XYZ	H1	#	#	#	Needle in azeotrope vessel
620	Dilutor	Dispense	L	750	20	N	Adding resolubilized/dry fluoride
640	Dilutor	Aspirate	L	900	5	N	Pull up 0.3 ml resolubilizing solvent
660	Dilutor	Dispense	L	1000	20	N	Pull up 0.3 ml resolubilizing solvent
680	Dilutor	Aspirate	L	900	5	N	Pull up 0.3 ml resolubilizing solvent
700	Dilutor	Dispense	L	1100	10	N	Pull up 0.3 ml resolubilizing solvent
720	Dilutor	Aspirate	L	1200	5	N	Pull up 0.3 ml resolubilizing solvent
740	Robot	Move_XYZ	H5	#	#	#	Needle in azeotrope vessel
750	Dilutor	Dispense	L	1200	5	N	Adding resolubilized/dry fluoride
760	Flow	Set_Flow	1	200	200	#	Setting nitrogen flow to 200 ml/min
770	Dout	On	29	#	#	#	Sterile needles go down
780	Dout	On	43	#	#	#	Sterile needles go down
790	#	Delay	3	#	#	#	blowing out residual MeCN
800	#	Attention	Perform 1st microwave irradiation for drying click OK when done	#	#	#	Fluoride vial added to Synthia rack
820	Dout	Off	29	#	#	#	
840	Dout	Off	43	#	#	#	
860	Flow	Set_Flow	1	0	200	#	Setting nitrogen flow to 200 ml/min
890	Dout	On	40	#	#	#	
900	#	Delay	2	#	#	#	
920	Dout	Off	26	#	#	#	
940	#	Delay	2	#	#	#	
950	Dout	Off	40	#	#	#	
960	Flow	Set_Flow	1	0	200	#	Setting nitrogen flow to 20 ml/min
970	Dout	Off	30	#	#	#	stop N2 flow in azeotrope vessel

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: Draft 8/17/2009

980	Flow	Set_Flow	1	10	200	#	Setting nitrogen flow to 20 ml/min
1000	#	Print	Transfering_Activity 2	#	#	#	
1020	Robot	Move_XYZ0	F16	#	#	#	Needle in Rxn vessel
1040	Dilutor	Aspirate	L	800	5	N	Adding resolubilized/dry fluoride
1060	Robot	Move_XYZ	F16	#	#	#	Needle in Rxn vessel
1080	Dilutor	Aspirate	L	700	5	N	Pull up 0.3 ml resolubilizing solvent
1100	Robot	Move_XYZ	H1	#	#	#	Needle in azeotrope vessel
1120	Dilutor	Dispense	L	750	20	N	Adding resolubilized/dry fluoride
1140	Dilutor	Aspirate	L	800	5	N	Pull up 0.3 ml resolubilizing solvent
1160	Dilutor	Dispense	L	1000	20	N	Pull up 0.3 ml resolubilizing solvent
1180	Dilutor	Aspirate	L	800	5	N	Pull up 0.3 ml resolubilizing solvent
1200	Dilutor	Dispense	L	1100	10	N	Pull up 0.3 ml resolubilizing solvent
1220	Dilutor	Aspirate	L	1200	5	N	Pull up 0.3 ml resolubilizing solvent
1240	Robot	Move_XYZ	H5	#	#	#	ASPEC blow needle in v-vial
1260	Dilutor	Dispense	L	1200	5	N	Adding resolubilized/dry fluoride
1280	#	Print	2 nd MW Drying	#	#	#	
1300	Dout	On	29	#	#	#	N2 path on
1320	Dout	On	43	#	#	#	N2 path on
1340	Flow	Set_Flow	1	200	200	#	Setting nitrogen flow to 200 ml/min
1360	#	Attention	Perform 2nd microwave irradiation for drying click OK when done	#	#	#	Fluoride vial added to Synthia rack
1400	Robot	Attention	Cooling for 2 minutes	#	#	#	Needle in fresh MeCN vial
1420	#	Delay	120	#	#	#	Trap needles go up
1430	Dout	Off	29	#	#	#	Trap needles go up
1440	Dout	Off	43	#	#	#	Trap needles go up
1450	Flow	Set_Flow	1	200	0	#	Setting nitrogen flow to 20 ml/min
2600	#	Print	Transfering_precursor	#	#	#	
2610	Robot	Move_XYZ0	F13	#	#	#	Needle in Rxn vessel
2620	Dilutor	Aspirate	L	500	5	N	Adding resolubilized/dry fluoride

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National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: Draft 8/17/2009

2630	Robot	Move_XYZ	F13	#	#	#	Needle in Rxn vessel
2640	Dilutor	Aspirate	L	1000	5	N	Pull up 0.3 ml resolubilizing solvent
2660	Robot	Move_XYZ	H5	#	#	#	Needle in azeotrope vessel
2680	Dilutor	Dispense	L	1300	5	N	Adding resolubilized/dry fluoride
2700	Robot	Move_XYZ0	A1	#	#	#	Needle in fresh MeCN vial
2720	#	Attention	Remove Vent needle. Click OK when done and perform reaction	#	#	#	Wait 2 s
2740	#	Print	Perform microwave irradiation	#	#	#	
2760	#	Print	Rinsing needle w/water	#	#	#	
2780	Robot	Move_XYZ0	B16	#	#	#	Needle in fresh MeCN vial
2800	Dilutor	Aspirate	L	1000	5	N	Pull up MeCN rinse of fluoride vessel
2900	Robot	Move_XYZ0	A1	#	#	#	Position over v-vial no reference
3000	Dilutor	Aspirate	L	4000	40	R	Asp. Dilution volume
3025	Dilutor	Dispense	L	4000	40	N	Disp. dilution vol
3050	Dilutor	Aspirate	L	4000	40	R	Asp. Dilution volume
3075	Dilutor	Dispense	L	4000	40	N	Disp. dilution vol
3100	Dilutor	Aspirate	L	5000	20	R	Asp. Dilution volume
3125	Dilutor	Dispense	L	4000	20	N	Disp. dilution vol
3130	#	Attention	Click OK when reaction done	#	#	#	Wait 2 s
3150	#	Print	Diluting the reaction mixture with 700uL water	#	#	#	
3160	Robot	Move_XYZ	H4	#	#	#	Position reaction vial in oven
3165	Dilutor	Dispense	L	700	10	N	Disp. dilution vol
3170	Robot	Move_XYZ0	H4	#	#	#	Position reaction vial in oven
3175	Dilutor	Aspirate	L	250	5	N	Pull up 0.3 ml resolubilizing solvent
3180	Robot	Move_XYZ	F7	#	#	#	Needle in Rxn vessel
3185	Dilutor	Aspirate	L	100	5	N	Pull up 0.3 ml resolubilizing solvent
3200	Robot	Move_XYZ0	H4	#	#	#	Position reaction vial in oven
3210	Dilutor	Aspirate	L	700	10	N	Aspirating air plug
3220	Robot	Move_XYZ	H4	#	#	#	Position reaction vial in oven
3230	Dilutor	Aspirate	L	1000	10	N	Aspirating air plug

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National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: Draft 8/17/2009

3240	Dilutor	Dispense	L	1500	10	N	Disp. dilution vol
3250	Dilutor	Aspirate	L	1500	10	N	Aspirating air plug
3260	Dilutor	Dispense	L	1500	10	N	Disp. dilution vol
3270	Dilutor	Aspirate	L	2000	5	N	Aspirating air plug
3280	#	Print	Injecting on the preparative HPLC system.	#	#	#	
3400	Robot	Move_XYZ	B11	#	#	#	Position preparative injection port
3500	Robot	Switch_Valve	P	L	#	#	Preparative injection valve set on Load
3550	Dilutor	Dispense	L	2200	5	N	Load reaction mixture + dil. Volume on prep injection valve
3600	#	Delay	5	#	#	#	
3650	Robot	Switch_Valve	P	I	#	#	Preparative injection valve set on Inject
3750	#	Print	Starting the preparative HPLC system.	#	#	#	
3800	Dout	On	56	#	#	#	Signalling to start prep HPLC system
4100	#	Delay	5	#	#	#	Wait 5 s (for system to start)
4200	Dout	Off	56	#	#	#	Stop signalling to prep HPLC
4800	#	Print	Rinsing the needle.	#	#	#	
4900	Robot	Move_XYZ	A1	#	#	#	Position rinsing needle
5000	Dilutor	Init	L	N	#	#	Initiating the dilutor
5100	Dilutor	Aspirate	L	5000	50	R	Rinsing the needle
5200	Dilutor	Dispense	L	5000	20	N	Rinsing the needle
5300	Robot	Move_XYZ	A3	#	#	#	Position rinsing the needle on the outside
5400	Dilutor	Aspirate	L	5000	50	R	Rinsing the needle
5405	Dilutor	Dispense	L	5000	20	N	Rinsing the needle
5410	Robot	Move_XYZ0	A1	#	#	#	Needle in fresh MeCN vial
5415	Flow	Set_Flow	1	50	200	#	Setting nitrogen flow to 20 ml/min
5420	Dout	On	29	#	#	#	Trap needles go up
5425	Dout	On	43	#	#	#	Trap needles go up
5430	Flow	Set_Flow	1	200	200	#	Setting nitrogen flow to 20 ml/min
5435	#	Delay	30	#	#	#	Trap needles go up
5440	Dout	Off	29	#	#	#	Trap needles go up
5445	Dout	Off	43	#	#	#	Trap needles go up

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National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: Draft 8/17/2009

5450	Flow	Set_Flow	1	0	200	#	Setting nitrogen flow to 200 ml/min
5480	Dilutor	Init	L	N	#	#	Initiating the dilutor
5500	Robot	Move_XYZ0	F28	#	#	#	Position rinsing the needle on the outside
5510	Dilutor	Aspirate	L	1000	10	N	Rinsing the needle
5515	Robot	Move_XYZ	F28	#	#	#	Position rinsing the needle on the outside
5520	Dilutor	Aspirate	L	5000	20	N	Rinsing the needle
5525	Robot	Move_XYZ	A3	#	#	#	Position rinsing needle
5530	Dilutor	Dispense	L	5000	20	N	Rinsing the needle
5535	Robot	Move_XYZ	F31	#	#	#	Position rinsing the needle on the outside
5540	Dilutor	Aspirate	L	6000	30	N	Rinsing the needle
5545	Robot	Move_XYZ0	A1	#	#	#	Position rinsing needle
5550	Dilutor	Dispense	L	6000	20	N	Rinsing the needle
5551	Robot	Move_XYZ	F4	#	#	#	Position rinsing needle
5552	Dilutor	Aspirate	L	5000	20	N	Rinsing the needle
5553	Robot	Move_XYZ0	A1	#	#	#	Position rinsing needle
5554	Dilutor	Dispense	L	5000	10	N	Rinsing the needle
5555	Robot	Move_XYZ	F14	#	#	#	Position rinsing needle
5560	Dilutor	Aspirate	L	1500	20	N	Rinsing the needle
5565	Robot	Move_XYZ0	A1	#	#	#	Position rinsing needle
5570	Dilutor	Dispense	L	1500	10	N	Rinsing the needle
5575	Robot	Move_XYZ	F15	#	#	#	Position rinsing needle
5580	Dilutor	Aspirate	L	1500	20	N	Rinsing the needle
5585	Robot	Move_XYZ0	A3	#	#	#	Position rinsing needle
5590	Dilutor	Dispense	L	1500	10	N	Rinsing the needle
5595	Robot	Move_XYZ0	A1	#	#	#	Position rinsing needle
5596	Dilutor	Aspirate	L	8000	30	R	Rinsing the needle
5597	Dilutor	Dispense	L	8000	20	N	Rinsing the needle
5600	Oven	Set_Temperature	7	18	#	#	slowly decreasing oven temp
5700	Robot	Move_XYZ	J99	#	#	#	Position prep tube over prep position 5
5720	#	Ask	Collect fraction in large test tube?	Not_4	#	#	
5730	Dout	On	41	#	#	#	Collecting prep fraction
5740	#	Print	Collecting fraction in large test tube.	#	#	#	
5750	#	Attention	Press Ok to stop collecting in large test tube.	#	#	#	
5760	Dout	Off	41	#	#	#	Stop collecting prep fraction

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Bethesda, MD 20892

Date of review: Draft 8/17/2009

6230	#	Attention	Synthesis is finished : perform Formulation	#	#	#	
6280	Oven	Set_Temperature	6	18	#	#	Turn off reaction oven
6320	Oven	Set_Temperature	8	18	#	#	Turn off evaporator oven
6340	Robot	Home	#	#	#	#	Position home
6350	Vacuum	Stop	#	#	#	#	Turn off vacuum pump
7000	Dout	Reset_All	#	#	#	#	Set all Dout devices to Off

FORMULATION

Row_nr	Object	Function	Arg_1	Arg_2	Arg_3	Arg_4	Comments
1	Dout	Reset_All	#	#	#	#	Set all Dout devices to Off
5	#	Delay	2	#	#	#	Wait 3 s
10	Robot	Home	#	#	#	#	Position home
15	#	Delay	2	#	#	#	Wait 3 s
20	#	Print	Positioning the SPE slider.	#	#	#	
25	Robot	Move_XYZ	I1	#	#	#	Positioning the SPE slider
30	Robot	Move_XY	I6	800	#	#	Positioning the SPE slider
35	Flow	Set_Flow	2	0	200	#	Setting He flow to 0 ml/min
36	Robot	Move_XYZ0	A1	#	#	#	Position Needle at position A1
38	Flow	Set_Flow	1	50	200	#	Setting nitrogen flow to 20 ml/min
40	Dout	On	29	#	#	#	Trap needles go up
42	Dout	On	43	#	#	#	Trap needles go up
44	Flow	Set_Flow	1	200	200	#	Setting nitrogen flow to 20 ml/min
45	#	Delay	30	#	#	#	Trap needles go up
46	Dout	Off	29	#	#	#	Trap needles go up
48	Dout	Off	43	#	#	#	Trap needles go up
50	Flow	Set_Flow	1	0	200	#	Setting nitrogen flow to 200 ml/min
52	Dilutor	Init	L	N	#	#	Initiating the dilutor
55	#	Print	Rinsing the needle with EtOH	#	#	#	
58	Dilutor	Aspirate	L	1000	5	N	Pull up MeCN rinse of fluoride vessel
60	Robot	Move_XYZ	E13	#	#	#	Position rinsing needle
65	Dilutor	Aspirate	L	2500	10	N	Rinsing the needle
70	Robot	Move_XYZ0	A1	#	#	#	Position rinsing needle
75	Dilutor	Dispense	L	2500	10	N	Rinsing the needle
80	Robot	Move_XYZ	E12	#	#	#	Position rinsing needle

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Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: Draft 8/17/2009

85	Dilutor	Aspirate	L	2500	10	N	Rinsing the needle
90	Robot	Move_XYZ0	A1	#	#	#	Position rinsing needle
95	Dilutor	Dispense	L	3000	30	N	Rinsing the needle
100	#	Attention	Ready to start formulation?	#	#	#	
130	#	Print	Passing fraction through Sep Pak	#	#	#	
130	Dilutor	Init	L	N	#	#	Initiating the dilutor
200	Robot	Move_XYZ0	F99	#	#	#	Moving to large test tube
300	Dilutor	Aspirate	L	7000	15	R	Priming the dilutor / Rinse needle
400	Dilutor	Dispense	L	7000	25	N	Adding 7 ml to large tube
500	Dilutor	Aspirate	L	200	10	N	Aspirate air gap
600	Robot	Move_XYZ	F99	#	#	#	
610	Dilutor	Aspirate	L	9000	25	N	Aspirate solution to formulate
620	Robot	Move_XYZ	E29	#	#	#	Moving to seppak
630	Dilutor	Dispense	L	9000	5	N	Dispense solution to seppak
640	Flow	Set_Flow	1	20	200	#	Setting nitrogen flow to 20 ml/min
650	Dout	On	29	#	#	#	
660	Dout	On	43	#	#	#	
670	#	Delay	25	#	#	#	
680	Flow	Set_Flow	1	0	200	#	Setting nitrogen flow to 0 ml/min
690	Dout	Off	29	#	#	#	
695	Dout	Off	43	#	#	#	
800	Robot	Move_XYZ	F99	#	#	#	Moving to large test tube
810	Dilutor	Aspirate	L	9000	25	N	Aspirate solution to formulate
820	Robot	Move_XYZ	E29	#	#	#	Moving to seppak
830	Dilutor	Dispense	L	9000	5	N	Dispense solution to seppak
840	Flow	Set_Flow	1	20	200	#	Setting nitrogen flow to 20 ml/min
850	Dout	On	29	#	#	#	
860	Dout	On	43	#	#	#	
870	#	Delay	35	#	#	#	
880	Flow	Set_Flow	1	0	200	#	Setting nitrogen flow to 20 ml/min
890	Dout	Off	29	#	#	#	
900	Dout	Off	43	#	#	#	
1000	Robot	Move_XYZ	F99	#	#	#	Moving to large test tube
1010	Dilutor	Aspirate	L	9000	25	N	Aspirate solution to formulate
1020	Robot	Move_XYZ	E29	#	#	#	Moving to seppak

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Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
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Bethesda, MD 20892

Date of review: Draft 8/17/2009

1030	Dilutor	Dispense	L	9000	5	N	Dispense solution to seppak
1300	Dilutor	Aspirate	L	8000	25	R	Aspirate 8 ml water
1400	Dilutor	Dispense	L	8000	5	N	Dispense water to seppak
1500	Flow	Set_Flow	1	30	200	#	Setting nitrogen flow to 30 ml/min
1600	Dout	On	29	#	#	#	
1700	Dout	On	43	#	#	#	
1800	#	Delay	40	#	#	#	
1900	Flow	Set_Flow	1	0	200	#	Setting nitrogen flow to 0 ml/min
2000	Dout	Off	29	#	#	#	
2100	Dout	Off	43	#	#	#	
2200	Robot	Move_XYZ	I7	#	#	#	Positioning the SPE slider
2300	Robot	Move_XY	I8	800	#	#	Positioning the SPE slider
2320	Dout	Reset_All	#	#	#	#	Set all Dout devices to Off
2350	Robot	Move_XYZ0	F32	#	#	#	Moving to Alcohol solution
2410	Dilutor	Aspirate	L	500	10	N	Priming the dilutor / Rinse needle
2430	Robot	Move_XYZ	F32	#	#	#	
2440	Dilutor	Aspirate	L	2000	20	N	Aspirate 2 ml ethanol
2450	Robot	Move_XYZ0	F1	#	#	#	
2500	Dilutor	Dispense	L	2000	5	N	Dispense 2 ml ethanol
2600	Robot	Move_XYZ	F17	#	#	#	
2700	Dilutor	Aspirate	L	900	20	N	Aspirate 0.9 ml ethanol
2800	Robot	Move_XYZ	E20	#	#	#	
2900	Dilutor	Dispense	L	900	5	N	Dispense 0.9 ml ethanol
3000	#	Print	Aspirating the needle free from eluent.	#	#	#	
3500	Flow	Set_Flow	1	20	200	#	Setting nitrogen flow to 20 ml/min
3600	Dout	On	29	#	#	#	Trap needles go up
3700	Dout	On	43	#	#	#	Trap needles go up
3800	#	Delay	60	#	#	#	
3900	Flow	Set_Flow	1	0	200	#	Setting nitrogen flow to 0 ml/min
4000	Dout	Off	29	#	#	#	Trap needles go up
4100	Dout	Off	43	#	#	#	Trap needles go up
4200	#	Print	Positioning SPE slider	#	#	#	
4300	Robot	Move_XYZ	I1	#	#	#	Positioning the SPE slider
4400	Robot	Move_XY	I6	800	#	#	Positioning the SPE slider

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Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: Draft 8/17/2009

4600	Dilutor	Init	L	N	#	#	Initiating the dilutor
4650	#	Print	Transferring formule to dose	#	#	#	
4900	Robot	Move_XYZ0	F9	#	#	#	Moving to saline solution
5000	Dilutor	Aspirate	L	1000	40	R	Aspirate 0.5 ml saline solution
5200	Robot	Move_XYZ	F9	#	#	#	Moving to saline solution
5300	Dilutor	Aspirate	L	4000	5	N	Aspirate 4 ml saline solution
5400	Robot	Move_XYZ	F17	#	#	#	Moving to Ethanol USP
5450	Dilutor	Dispense	L	50	5	N	Dispense 100 ul ethanol
5500	Dilutor	Aspirate	L	150	5	N	Aspirate 0.1 ml Ethanol USP
5700	Robot	Move_XYZ	E5	#	#	#	Moving to Radioligand
5800	Dilutor	Dispense	L	50	5	N	Dispense 100 ul ethanol
5900	Dilutor	Aspirate	L	1000	5	N	Aspirate Radioligand
6000	Robot	Move_XYZ	B15	#	#	#	Move to Formulation port
6010	Robot	Switch_Valve	P	L	#	#	Preparative injection valve set on Load
6020	Dilutor	Dispense	L	5500	5	N	Load reaction mixture + dil. Volume on prep injection valve
6030	#	Delay	20	#	#	#	
6040	Robot	Switch_Valve	P	I	#	#	Preparative injection valve set on Inject
6200	Robot	Move_XYZ0	A1	#	#	#	Go to Initial position
6300	#	Attention	Stop Synthesis and perform QC	#	#	#	
6400	Robot	Move_XYZ	A1	#	#	#	Position A1
6500	#	Print	Rinsing the needle.	#	#	#	
6600	Dilutor	Aspirate	L	2000	50	R	
6700	Dilutor	Dispense	L	2000	20	N	
6800	Robot	Move_XYZ	A3	#	#	#	
6900	Dilutor	Aspirate	L	500	50	R	Rinsing the needle
7000	Dilutor	Dispense	L	500	20	N	Rinsing the needle
7100	Dout	Reset_All	#	#	#	#	Set all Dout devices to Off
7300	Robot	Home	#	#	#	#	Position home

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Date of review: Draft 8/17/2009

Appendix C

Calculations Worksheet[9]

Radiopharmacy Dose Sheet for [^{18}F]FBR			
6	**Primary Chemist:**		
7	**Lab Phone:**	(301) 451 3917	
8	**Office Phone:**		
9	EOS Activity read from Whole Dose Vial		=C29
10	Date (mm/dd/yy)		
11	Batch # (FBR-yymmdd)		
12			
13	mCi/mL=	=B31*10	=C29
14	SPECIFIC RADIOACTIVITY (mCi/μmol) =	=B31/B26	=C29
15	μg/mL =	=B27/0.1	
16	Maximum allowable injection volume based on carrier	=B19/B15	Maximum injection volume is the lesser of the two numbers when calculated from maximum allowable carrier or maximum allowable impurity.
17	Maximum allowable injection volume based on impurity	=B20/B23	
18	Total Volume of Formulated [18F]FBR for Injection =		
19	Maximum allowable injected mass (μg)	10	
20	Maximum allowable injected impurity mass (μg)	1	
21			
22	**% Chemical purity**	=B32/(B32+B33)	
23	Concentration FBR equivalent impurity μg/mL =	= B28/0.1	
24	Enter MW of std:	395.4	
25	Enter slope of calibration curve, M (x=μmol, y= Peak area)		
26	μmol of carrier in injected 100 μL aliquot	=B32/B25	
27	μg of FBR in 100 μL aliquot	=B26*B24	
28	μg of FBR equivalent impurity in 100 μL aliquot	=B33/B25*B24	**ENTER Time of Radioactivity Measurement (hh:mm)**
29	**Enter activity in 100 μL hot aliquot (in mCi)**		
30	**Enter activity remaining in syringe after injection (mCi)**		
31	Net Activity (mCi) in 100 μL aliquot	=B29-B30	
32	**Enter Peak area of carrier in 100 μL aliquot**		
33	**Enter sum of all impurity Peak areas in 100μL aliquot**		
35	Second Activity Reading from Whole Dose		
36	Time (min) between first and second whole dose measurement		
37	Calculated Half Life	=0.693*B35/(LN(B9/B34))	

[9] Cells shaded gray are for input. All other cells in the worksheet are locked and pass word protected.

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Date of review: Draft 8/17/2009

Appendix D

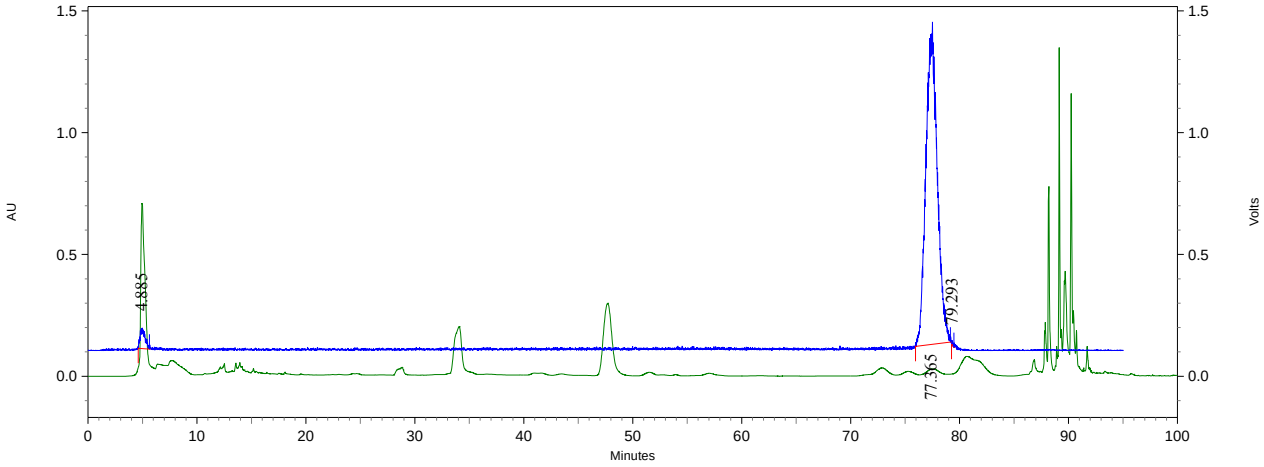
Representative Chromatograms

HPLC Data – Representative FBR Separation Chromatogram

FBR Preparative HPLC

Data File: D:\32Karat\Projects\PBR\Data\3-2007\FBR 070329 Prep
Method: D:\32Karat\Projects\PBR\methods\PBR Prep.met
Acquired: 3/29/2007 10:27:34 AM

Det 230 nm with BioScan

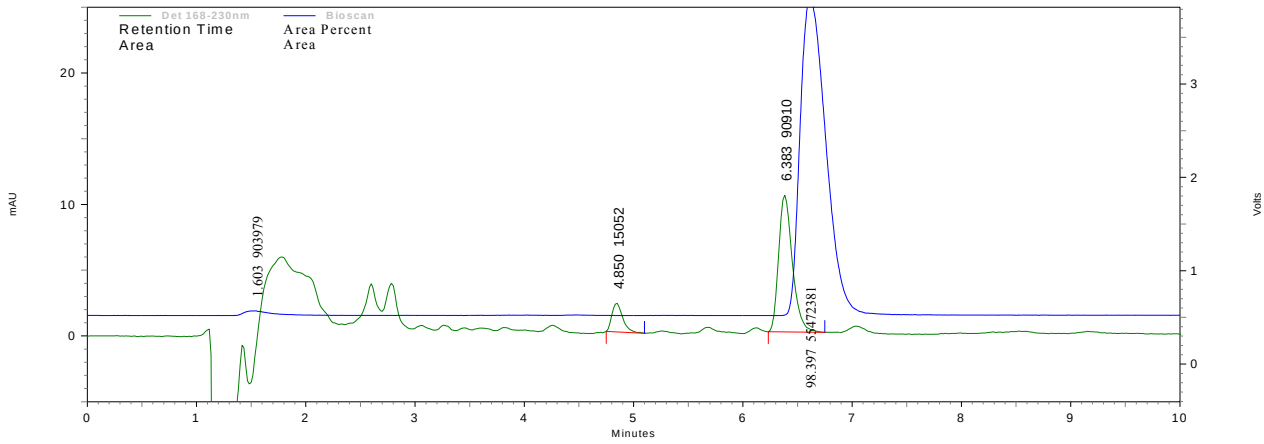


Note: HPLC traces provided as typical examples. Not all preparative traces will be identical.

HPLC Data – Representative FBR Analytical Chromatogram

FBR QC

Data File: D:\32Karat\Projects\Projects\FBR\Validation\04-06-2007\FBR070406 fd 100 uL inj.dat
Method: D:\32Karat\Projects\Projects\FBR\Method\FBR QC.met
Acquired: 4/6/2007 11:32:42 AM
HPLC: 10 mM Ammonium Formate in 60% MeCN (aq), 1.0 mL/ min Luna Hexyl-Phenyl 3 µm, 150 x 4.6 mm
Det 230 nm (note: UV and gamma detectors in series for this instrument and flow rate are approximately 0.5 minutes apart)



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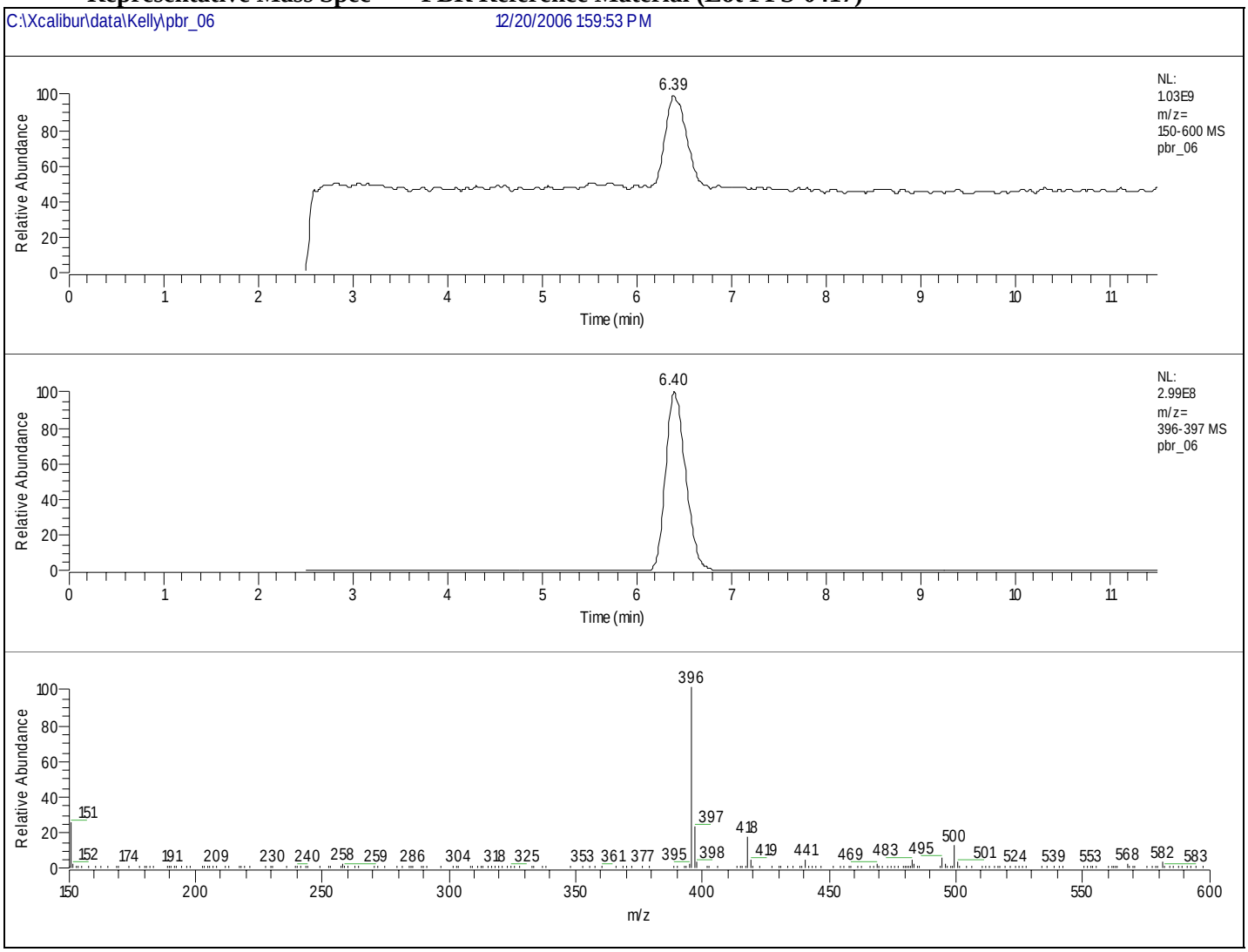
Date of review: Draft 8/17/2009

Appendix E

Characterization/ Acceptance Data **FBR Reference Material (Lot FPS-0417)**

Representative Mass Spec **FBR Reference Material (Lot FPS-0417)**



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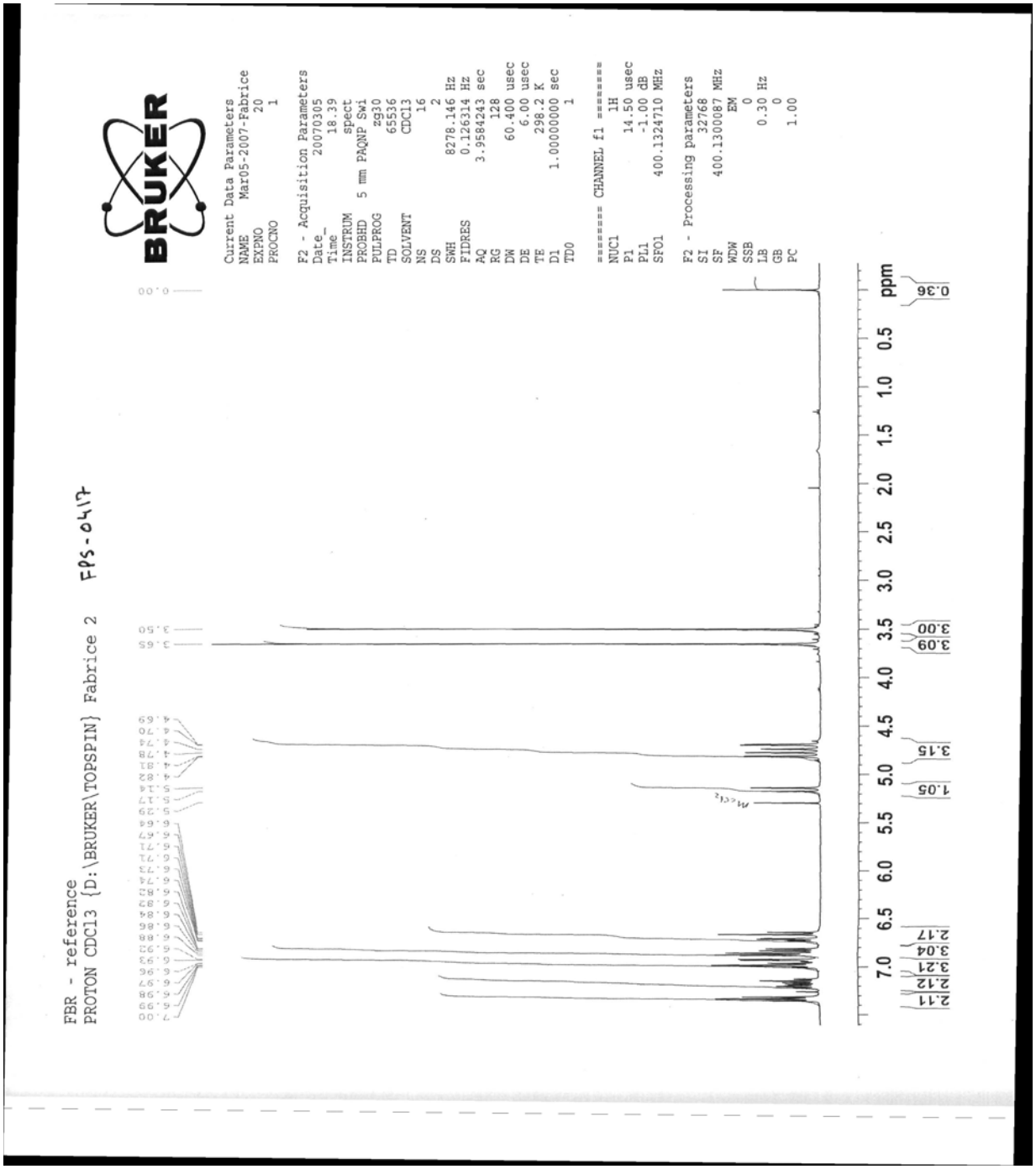
Date of review: Draft 8/17/2009

Reference NMR

^{1}HNMR **FBR Reference Material (Lot FPS-0416)**



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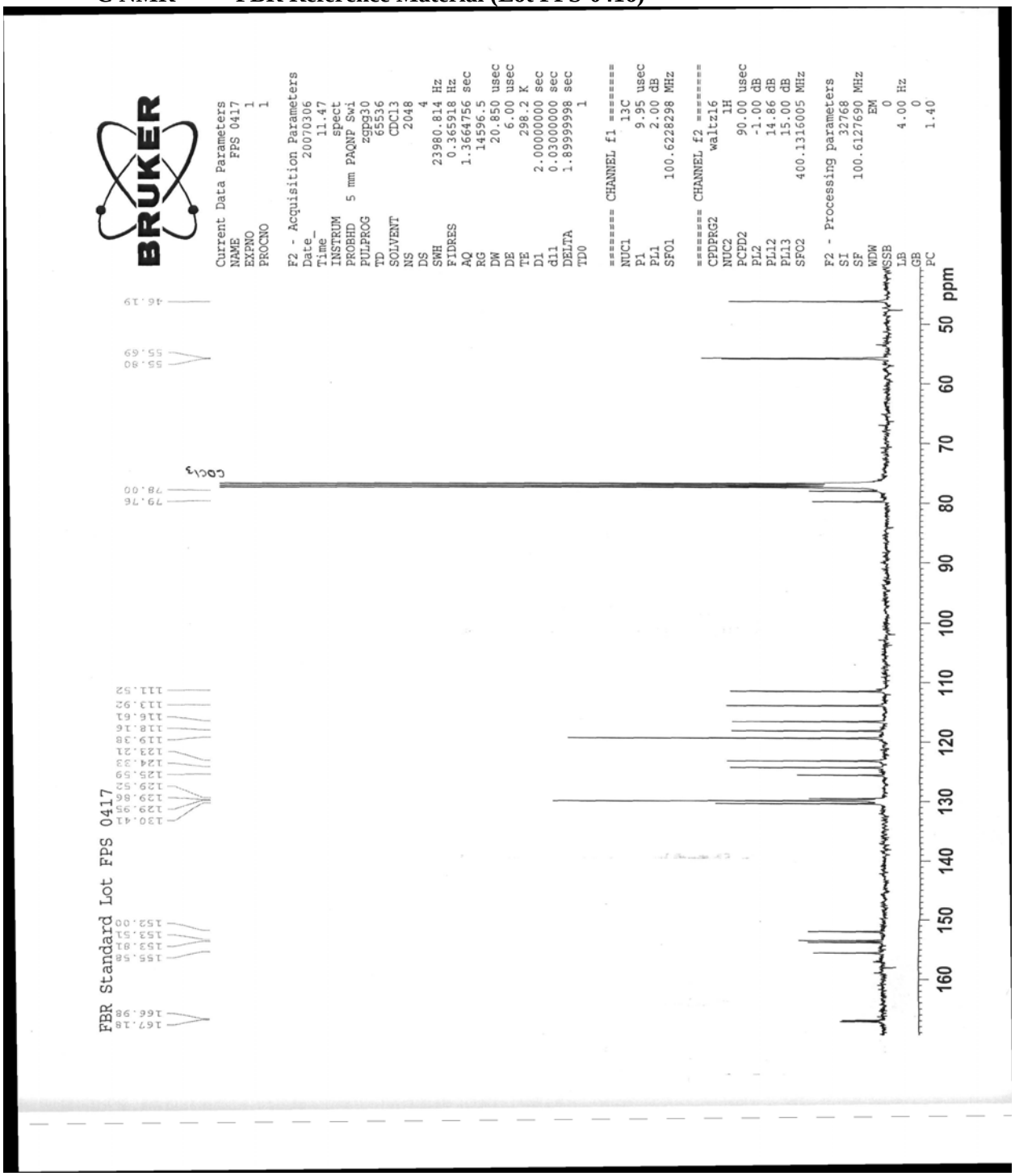
^{13}C NMR FBR Reference Material (Lot FPS-0416)



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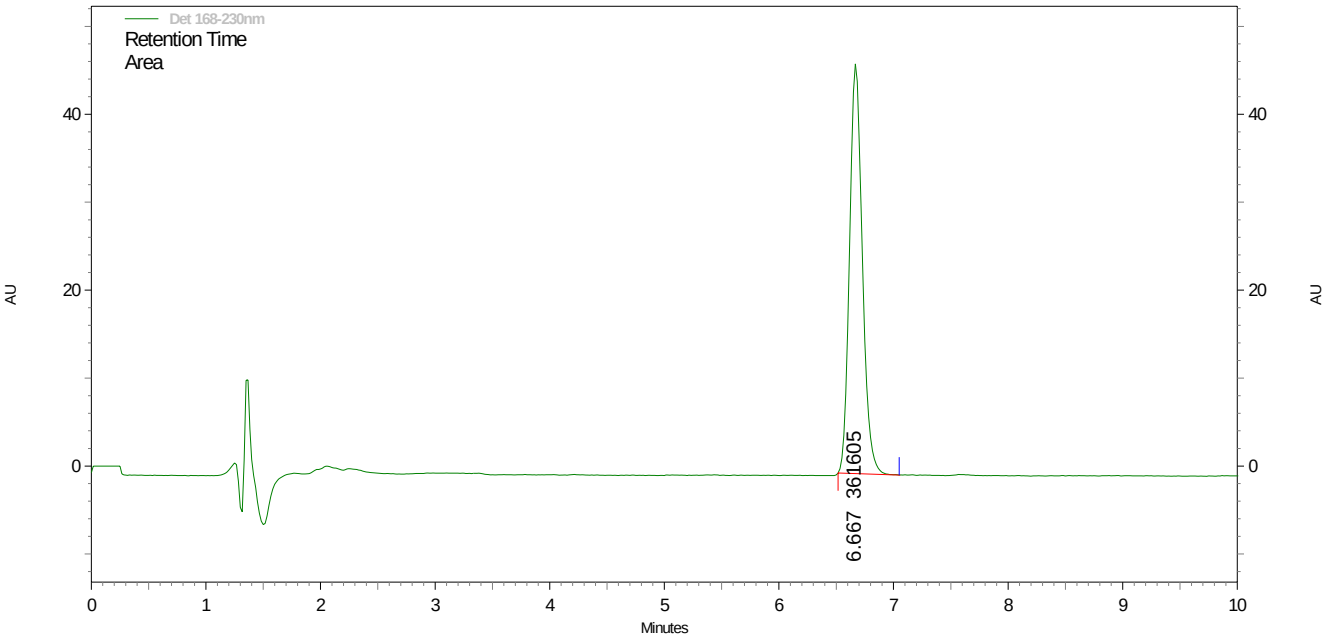
HPLC Purity FBR Reference Material (Lot FPS-0416)

FBR Standard Qualification

Data File: D:\32Karat\Projects\Projects\FBR\Data\Prevalidation\FBR test standard.dat
Method: D:\32Karat\Projects\Projects\PBR-06\Method\3 um Phenyl 60 percent MeCN 10 mM AF.met
Acquired: 2/6/2007 7:49:45 AM
HPLC: 10 mM Ammonium Formate in 60% MeCN (aq), 1.0 mL/ min Luna Hexyl-Phenyl 3 µm, 150 x 4.6 mm
230 nm



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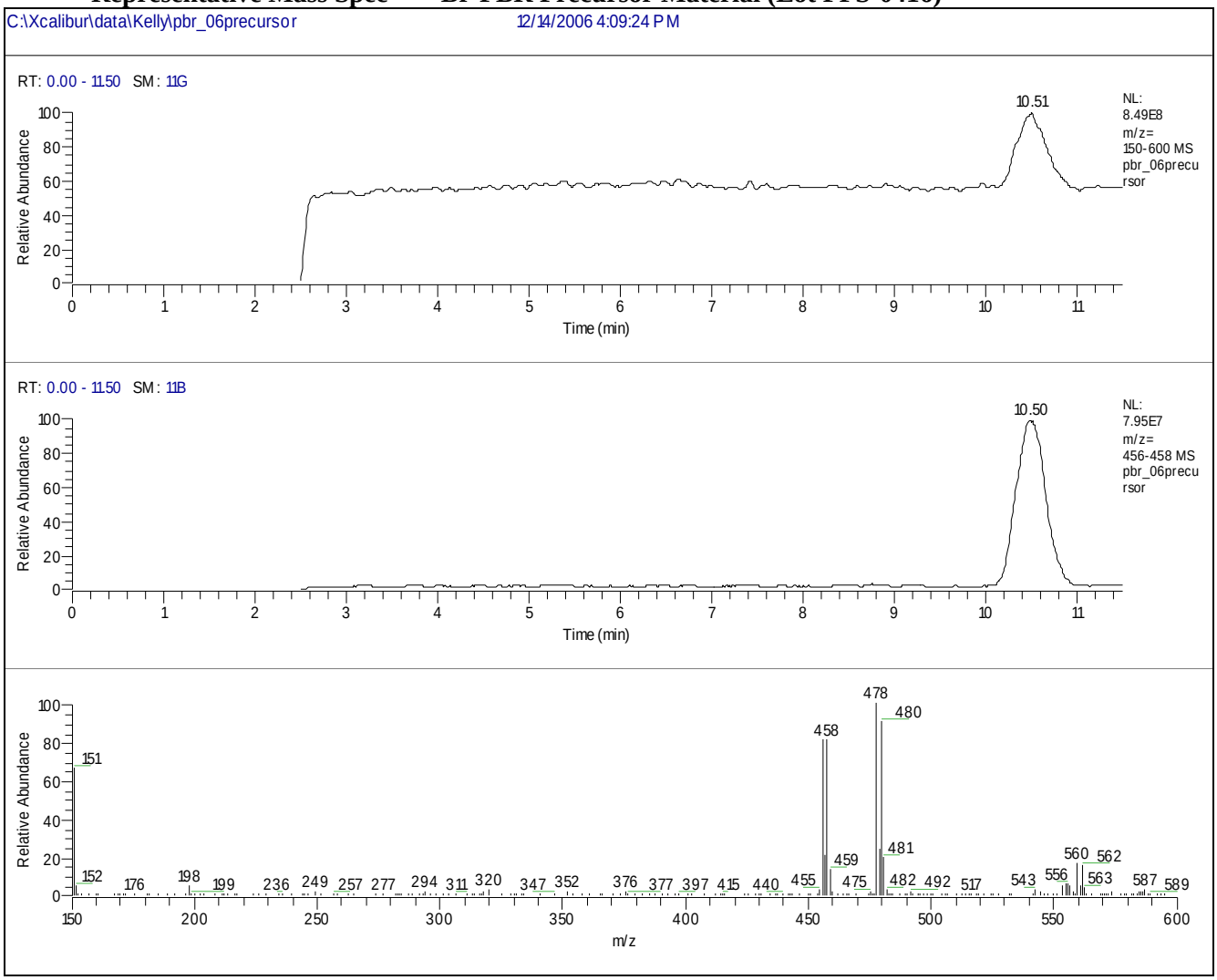
Appendix F

Characterization/ Acceptance Data — Br-FBR Precursor Material (Lot FPS-0416)

Representative Mass Spec — Br-FBR Precursor Material (Lot FPS-0416)



Reference NMR

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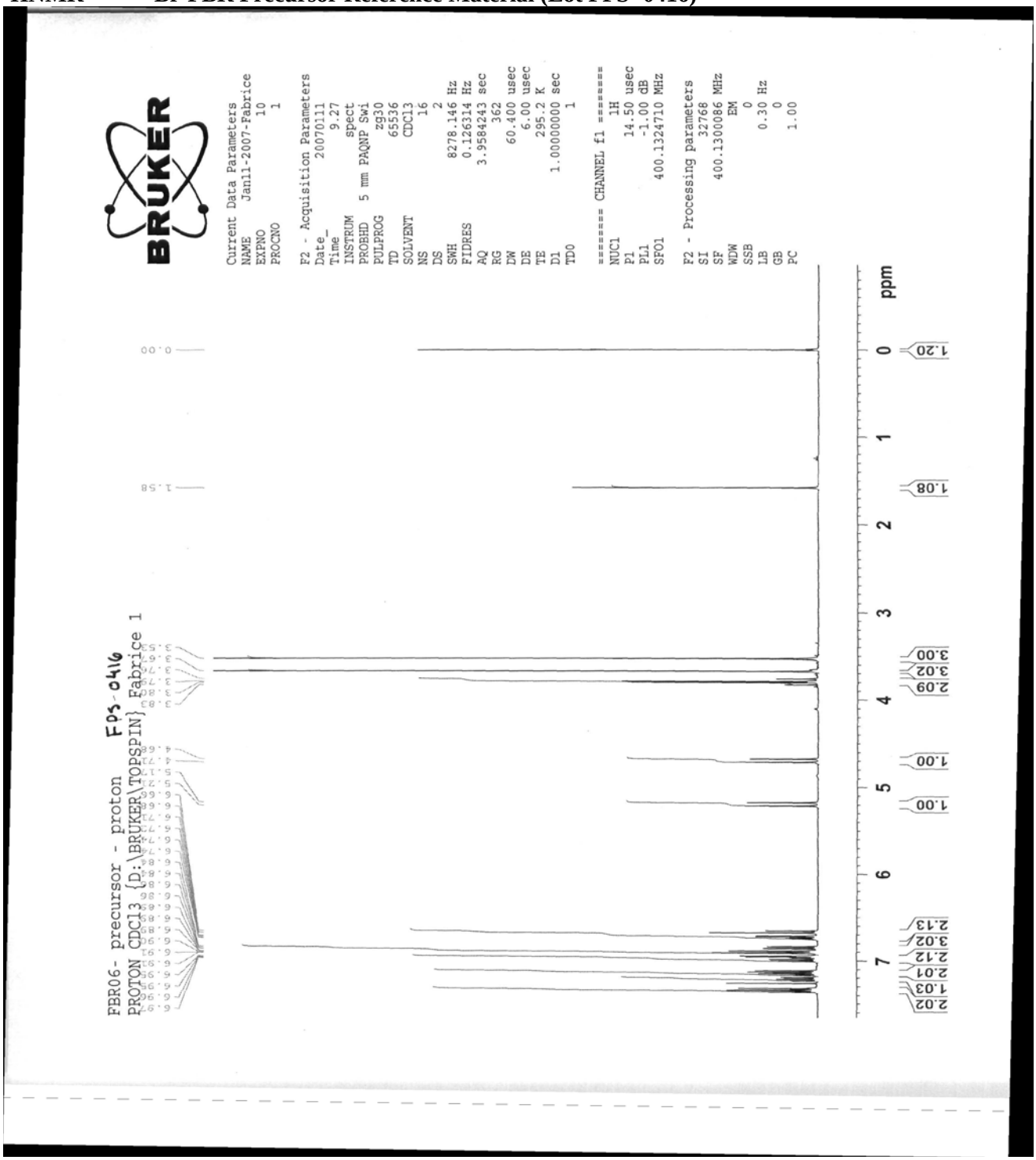
^{1}HNMR Br-FBR Precursor Reference Material (Lot FPS- 0416)



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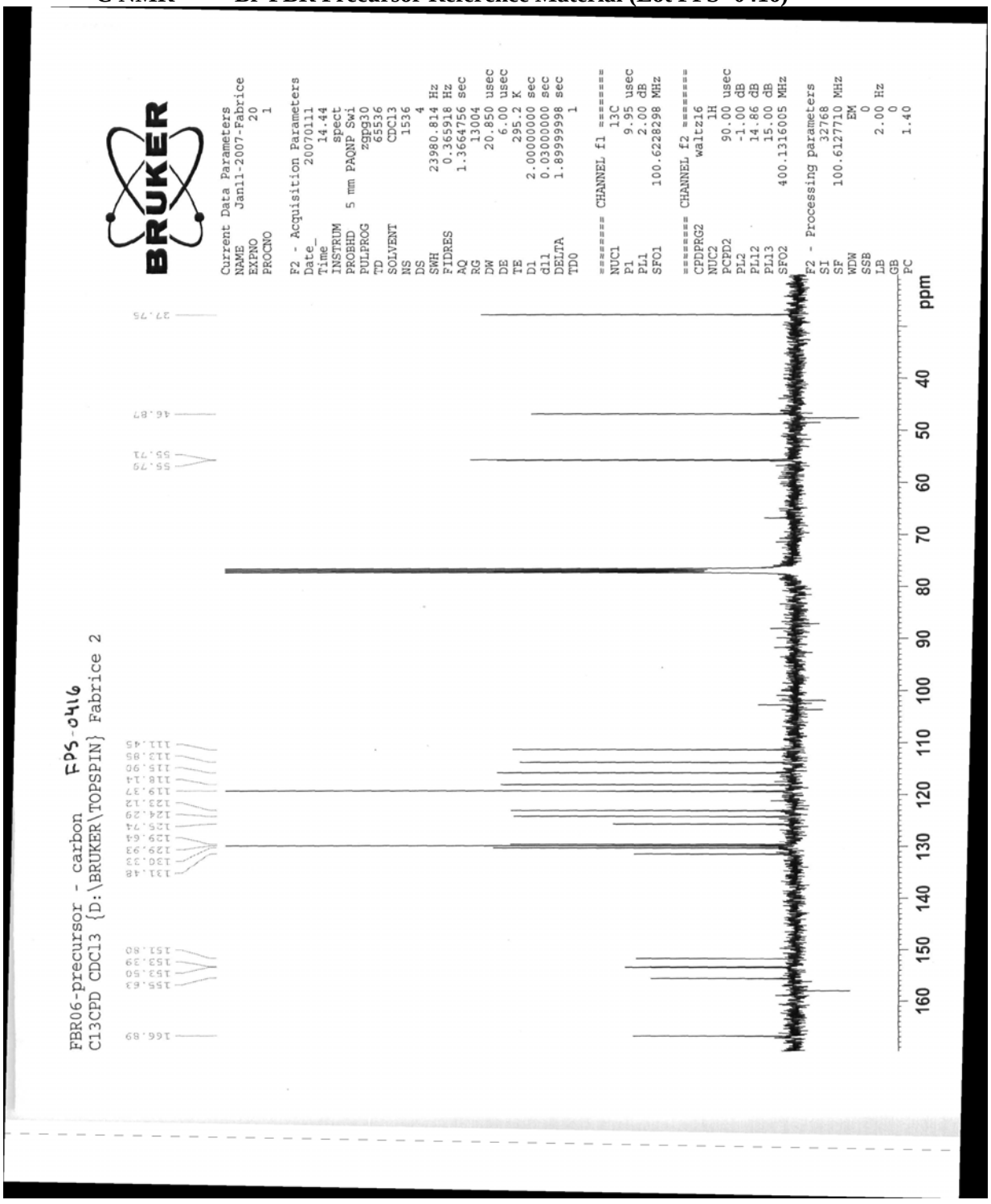
^{13}C NMR Br-FBR Precursor Reference Material (Lot FPS- 0416)



HPLC Purity Br-FBR Precursor (Lot FPS-0417)

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Br-FBR Acceptance

Data File: D:\32Karat\Projects\Projects\FBR\Acceptance Criteria\04-10-2007\FBR070410 FS 016 precursor 200 uL inj
Method: D:\32Karat\Projects\Projects\FBR\Method\FBR QC.met
Acquired: 4/10/2007 4:03:14 PM
HPLC: 10 mM Ammonium Formate in 60% MeCN (aq), 1.0 mL/ min Luna Hexyl-Phenyl 3 µm, 150 x 4.6 mm
230 nm