[^{11}C]ER176 Injection: Chemistry, Manufacturing and Controls

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: 2/18/2014

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1. DRUG PRODUCT COMPONENTS AND QUANTITATIVE COMPOSITION

Component	Composition/batch
Drug Substance* [^{11}C]ER176	20 to 250 mCi at EOS[1]
Other Ingredient(s) 0.9% Sodium Chloride, Injection, USP	10.0 mL
Ethanol, USP	0.5 ml

* Throughout this application [^{11}C]ER176 refers to the drug substance and *[^{11}C]ER176 Injection* to the final product

2. CONTROLS FOR COMPONENTS AND RAW MATERIALS

2.1. Organic Substrate (Key intermediate)

Name of Component	*N*-desmethyl-ER176; synonyms: (R)-N-sec-butyl-4-(2-chlorophenyl)quinazoline-2-carboxamide
Name and Address of Supplier	Dip. Scienze Farmaceutiche-Università di Pisa, Via Bonanno 6, 56126 Pisa
Certificate of Analysis (COA)	See Document 7
Storage Conditions	1. Container/Closure screw cap glass vial housed in a plastic container 2. Stored at 2-8 °C 3. The material is stable for at least one year, when stored in above container/closure under described conditions.

2.2. Target Starting Material

All the required carbon-11 is produced as [^{11}C]carbon dioxide at the NIH Cyclotron Facility.

The following target material will be used for the production of [^{11}C]carbon dioxide:

Name of the target material	Nitrogen gas with about 1% oxygen. Refer to Document 8 for COA.
Name and address of the target material manufacturer	Matheson Gas 932 Paterson Plank Road East Rutherford, NJ 07073
Identity test performed to release each lot for production use	COA
COA	A representative copy of supplier's COA is provided in COA Attachment Section
Target material recycling criteria	N/A

[1] EOS = End of Synthesis calibration time

2.3. Other Ingredients

Material	Specification	Supplier	Document 8 Page #
0.9% Sodium chloride	Injection, USP	Abraxis Pharmaceutical Products Schaumberg IL 60173	6
Dehydrated ethanol	Injection, USP	American Regent, Inc One Luitpold Drive P.O. Box 9001 Shirley NY 11967	7

2.4. Materials (Reagents, Solvents, Gases, Purification Columns, etc.)

Material	Specification	Supplier	Document 8 Page #
Dimethyl sulfoxide	99.9+%	Sigma-Aldrich PO Box 14508 St. Louis MO 63178	2
Potassium hydroxide	99.99%	Sigma-Aldrich PO Box 14508 St. Louis MO 63178	3
Water	HPLC grade	EMD Chemicals Inc. 480 S. Democrat Rd. Gibbstown NJ 00827 *or* Sigma-Aldrich PO Box 14508 St. Louis MO 63178	18
Acetonitrile	HPLC grade	Burdick & Jackson 1953 South Harvey Street Muskegon MI 49442 *or* Sigma-Aldrich PO Box 14508 St. Louis MO 63178	19
Methanol	HPLC grade	Fisher Scientific Inc. 47777 Warm Springs Blvd Fremont, CA 94539	17
Ammonium hydroxide, 1N	ACS grade	Ricca Chemical Company Arlington, TX 76012	20
Absolute ethanol, 200 proof	USP	Warner-Graham Co. P.O. Box 249, 160 Church Lane Cockeysville MD 21030	5
Sterile filter (Millex GV; 4 mm; 0.22 µm vent type)	Sterile	Millipore 80 Ashby Road Bedford MA 01730	13

Sterile filter (Millex MP; 25 mm; 0.22 µm)	Sterile	Millipore 80 Ashby Road Bedford MA 01730	14
HPLC column (Luna C18, 10 µm, 4.6 x 250 mm)	NA	Phenomenex	21
HPLC column (XTerra RP-18, 10 µm, 7.8 x 300 mm)	NA	Waters	22
Nitrogen gas	Ultra High Purity Carrier Grade	Roberts Oxygen Company, Inc. 15830 Redland Road Rockville MD 20855	26
Iodine (s)	≥ 99%	Fisher Scientific 2000 Park Lane Dr Pittsburgh PA 15275	28
Helium	99.9999%	Roberts Oxygen Company, Inc. 15830 Redland Road Rockville MD 20855	27
1% O_2 in N_2	Ultra High Purity	Roberts Oxygen Company, Inc. 15830 Redland Road Rockville MD 20855	25

3. REFERENCE STANDARDS

The following reference standard is used in the quality control of *[^{11}C]ER176 Injection*.

Material	Supplier	COA and Acceptance Criteria
ER176: synonyms: (R)-N-sec-butyl-4-(2-chlorophenyl)-N-methylquinazoline-2-carboxamide	Dip. Scienze Farmaceutiche-Università di Pisa, Via Bonanno 6, 56126 Pisa	Acceptance criteria: ^{1}H-NMR, ^{13}C-NMR, HPLC and LC-MS, Document 7

4. MANUFACTURING AND TESTING FACILITIES

Facility Address	Contact Persons
PET Radiopharmaceutical Sciences Section (PRSS), Molecular Imaging Branch (MIB), National Institute of Mental Health (NIMH), National Institutes of Health (NIH), Building 10, Room B3C342, 10 Center Drive, MSC 1003 Bethesda MD 20892	Victor W. Pike, Ph.D., Chief, PRSS Phone: (301) 596-5986 Yi Zhang, Ph.D, Radiochemist, PRSS

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5. MANUFACTURE OF DRUG SUBSTANCE

5.1. Batch Formula

The following is a list of the components and their quantities used in the production of each batch of *[^{11}C]ER176 Injection*.

Name of Component	Component Function	Amount Used
[^{11}C]Carbon dioxide	Radiolabeling reagent	0.1 – 3.0 Ci
N-Desmethyl-ER176	Radiopharmaceutical precursor	0.6 ± 0.06 mg
Dimethyl sulfoxide (anhydrous)	Reaction Solvent	0.4 mL
Potassium hydroxide	Reaction base	1.2 ± 0.2 mg
Water, HPLC grade	HPLC mobile phase component	At least 1 L
Acetonitrile, HPLC grade	HPLC mobile phase component	At least 1 L
Methanol, HPLC grade	HPLC mobile phase component	At least 740 mL
Ammonium hydroxide, 1N	HPLC mobile phase component	At least 1 mL
HPLC column, 10 x 250 mm	Purification of product	1
0.9% Sodium chloride for injection, USP	Formulation component	10.0 mL
Ethanol, dehydrated	Formulation component	0.5 mL
Absolute ethanol, 200 proof	Cleaning of rotary evaporator and Synthia apparatus	As needed
Sterile empty vial, 10 mL	Product container	1
Sterile filter (Millex MP, 0.22 µm; 25 mm)	Sterile filtration of product	1
Sterile vent filter (Millex GV, 0.22 µm; 4 mm)	Sterile vent filter	1
Sterile needle, 20G x 1½”	Product transfer vent needle	1
Sterile needle, 21G x 2”	Product transfer, QC sampling and [^{11}C]Iodomethane transfer	3
Sterile syringe, 1 mL	QC sampling	1

NOTE: Upon scale-up, only the amount of radioactive [^{11}C]carbon dioxide reagent is changed. The other components and their amounts remain as stated in the batch formula.

5.2. Production of Radionuclide

All [^{11}C]carbon dioxide is prepared at the NIH Cyclotron Facility. No other source of [^{11}C]carbon dioxide is used for the production of *[^{11}C]ER176 Injection*.

5.2.1. Make and Model of Cyclotron

Any one of the following cyclotrons is used for the production of [^{11}C]carbon dioxide.

Manufacturer	Model
General Electric	PETtrace
Cyclotron Corporation	CS-30

5.2.2. Specifications for Target Body

Target Data	CS-30	GE PETtrace # 1	GE PETtrace # 2
Target body material	Aluminum	Aluminum	Aluminum
Entrance target foil material	Aluminum	Havar	Havar
Target length (cm)	25.4	25	25
Target volume (mL)	129	75	75
Gas pressure (atm)	17	10	10
Maximum proton energy (MeV)	20	16.5	16.5
Maximum beam current (μA)	25	50	50

5.3. Synthesis and Purification of the Drug Substance

5.3.1. Description of Radiosynthesis Equipment and Its Operation

Descriptions of the radiosynthetic equipment, its cleaning and operation are described in the SOPs for the unit: Document 2, SOPs #GP102, #MP201 and #MP202.

5.3.2. Radiosynthetic Production Unit

Operation Function	Manufacturer	Model	Serial #
Radiosynthesis	General Electric	Methyl Iodide Micro Lab	27740
Radiosynthesis	General Electric	Synthia	Cecelia - 001
HPLC purification	Beckman Coulter	System Gold 126 Solvent Module	342-2361
UV absorbance detection in HPLC purification	Beckman Coulter	System Gold 166 detector	322-2189
Radioactivity detection in HPLC purification	Bioscan	Flow Count	0605-317

5.3.3. In-Process Controls

The Methyl Iodide Micro Lab module and Synthia apparatus continuously record data from transducers during the production process. The data displays are monitored by the operator during the production, and the process is also monitored visually by the operator to ensure that all steps proceed according to the recipe.

5.3.4. Post-Synthesis Procedures

Descriptions of procedures used to prepare the production equipment, including any cleaning and purging procedures for a subsequent batch, are provided in Document 2, SOPs #GP102, #GP103, #MP201 and #MP202.

6. MANUFACTURE OF DRUG PRODUCT

6.1. Production Operation

The drug substance, [^{11}C]ER176, is prepared using an automated Synthia synthesis apparatus coupled with an automated GM Methyl Iodide MicroLab synthesis apparatus. The synthesis and purification of the drug product involves the following steps:

6.1.1. The production operation is initiated by manually loading N-desmethyl-ER176 (0.6 ± 0.06 mg) and potassium hydroxide (1.2 ± 0.2 mg) dissolved in dimethyl sulfoxide (0.4 mL) into Slider Position 2 of the Synthia apparatus.

6.1.2. [^{11}C]Carbon dioxide, produced from the cyclotron, is then converted into [^{11}C]iodomethane via the GE MicroLab module. The [^{11}C]iodomethane is then transferred into the Synthia apparatus and reacted with the *N*-desmethyl-ER176 to produce [^{11}C]ER176.

6.1.3. The crude reaction mixture is diluted with water (0.5 mL) and purified by HPLC on a Waters XTerra RP18 7.8 x 300 mm, 10 µm column, using an acetonitrile - 1 mM aqueous ammonium hydroxide isocratic method. The product is collected from the column into a rotary evaporator flask.

6.1.4. The isolated product is concentrated under reduced pressure and reconstituted with a solution of ethanol (USP, 0.5 mL) in 0.9 % Sodium Chloride for Injection (USP, 10 mL). The solution is passed through a sterile Millipore Millex MP filter (0.22 µm) and transferred directly into a sterile empty vial (10 mL size). The sterile empty vial is assembled with all needles and filters under aseptic conditions in a sterile cabinet (certified laminar flow cabinet in Room B3C349).

6.1.5. The exact production and quality control procedures used in the controlled production of *[^{11}C]ER176 Injection* are provided in Document 2: Standard Operating Procedures. Details of all materials and results for each batch are recorded on Document 3: Master Batch Record.

Attached for each batch of *[^{11}C]ER176 Injection* are the following:

Master Batch Record (Document 3)

- Form contains summary of materials used and batch results
- Preparative HPLC report

Quality Control Record (Document 4)

- Form contains summary of the pre-release quality control results
- Analytical HPLC report of formulated dose
- Analytical HPLC report of reference standard
- Gas Chromatography report

Post Release Test Record (Document 5)

- Form contains summary of post-release quality control results
- Sterility testing report

Calculations Worksheet

Any other ancillary data generated or collected that pertain to the specific batch of *[^{11}C]ER176 Injection*.

6.2. Reprocessing of Drug Product

The PRSS does not reprocess *[^{11}C]ER176 Injection.*

6.3. Packaging and Labeling

The components used in the packaging of the drug product vial and the method of labeling are described in Document 2: Standard Operating Procedures.

7. CONTAINER/CLOSURE

The pre-sterilized, pre-sealed, pyrogen-free container/closure is obtained from Hospira, Inc. Full information on the container/closure along with its contents, sterilization procedures, and sterility assurance are provided in the attached Certificate of Analysis (see Document 8).

Name and address of supplier	Hospira, Inc. 275 North Field Drive, Lake Forest, Illinois 60045
Container description	Sterile empty vial (nonpyrogenic, dry heat sterilized, no fluid)
NDC/List number	5816-11
Container	Flip-top – Vial - Glass (LF)
Representative COA	Document 8

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8. CONTROLS FOR THE FINISHED DOSAGE FORM

8.1. Sampling Procedures

Each batch of *[^{11}C]ER176 Injection* will be produced in one vial. The volume that is withdrawn from the finished drug product container and the distribution of this volume among individual tests are described in Document 2: Standard Operating Procedures, SOP #QA302.

8.2. Regulatory Specifications, Procedures and Tests

Each batch of *[^{11}C]ER176 Injection* will meet the following specifications during its entire shelf life (see below). We assure that any batch that fails to meet the acceptance criteria for release will not be released. We also assure that FDA will be notified of any changes to the approved application.

Note: The following tests are related to the production method proposed in this IND submission. In the event that the production method does not use a component listed, uses an alternative production method, or produces additional impurities not seen previously, a set of appropriate tests, acceptance criteria, procedures and a testing schedule that is more appropriate for such production will be proposed.

Test	Acceptance Criteria	Test Description	Testing Schedule
Appearance	Formulated dose is clear, colorless and free of particulates	Visual inspection	Completed before product release
Bacterial endotoxins (LAL)	NMT[2] 175 EU in injectable volume	EndoSafe®-PTS *Limulus* amebocyte lysate (LAL) test	Completed before product release
Chemical purity	Maximum volume contains NMT 5 µg of carrier and NMT 0.5 µg ER176 equivalent impurity	HPLC QC procedure	Completed before product release
Membrane filter integrity	No air passage through filter observed at 45 psi	Pressurize sterile filter above 45 psi to determine whether membrane has remained intact	Completed before product release
pH	pH 4.0 - 7.5	Measured using narrow range pH paper	Completed before product release
Radiochemical identity	Retention time within ± 1.0 min of a standard injection of ER176	HPLC QC procedure	Completed before product release
Radiochemical purity	NLT[3] 95% [^{11}C]ER176	HPLC QC procedure and LC/MS	Completed before product release
Radionuclidic identity	The measured half-life is 20.4 ± 2 min	Calculated half life from two measurements at least 3 minutes apart	Completed before product release

[2] Not More Than
[3] Not Less Than

Residual solvents	≤ 4.1 mg acetonitrile in the injectable dose[4] and ≤ $1x10^5$ ng/µL ethanol	Gas chromatography with flame ionization detection	Completed before product release
Specific radioactivity	NLT 500 mCi/µmol at EOS	HPLC QC procedure	Completed before product release
Sterility testing	No aerobic or anaerobic growth observed	NIH Clinical Center Microbiology Lab	Completed after product release

9. MICROBIOLOGICAL VALIDATION

Data provided in Document 9, Validation Record, show that *[^{11}C]ER176 Injection* is obtained in a sterile and pyrogen-free form when prepared as described in this application and the submitted batch production records.

10. STABILITY AND BATCH DATA

10.1. Expiry Dating Period

The expiry dating period is 1 h from the EOS calibration time of *[^{11}C]ER176 Injection* when the product is stored at controlled room temperature. Data provided in Document 9, Validation Record, show that *[^{11}C]ER176 Injection* is stable until the expiry time.

10.2. Stability Data/Batch Data

Complete release and stability data were obtained for three batches of *[^{11}C]ER176 Injection*, prepared and stored at controlled room temperature. See Document 9: Validation Record.

For each stability batch:

- The batch was stored in the same container/closure as it was produced.
- All tests indicated in the specification section were performed with satisfactory results before or immediately following release, as indicated.
- The appearance and radiochemical purity were evaluated at the end of the proposed expiry period with satisfactory results.

11. VIAL AND OUTER PACKAGING LABELS

Proposed vial and outer packaging labels are shown in Document 2: Standard Operating Procedures, SOP #QA301. Each batch will be labeled with a lot number, compound name, volume and quantitative assay and will contain the statement: "Caution: New Drug Limited by Federal Law to Investigational Use".

[4] Refer to USP <467>

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12. ENVIRONMENTAL ASSESSMENT

In accordance with 21 CFR 25.31(e), the PRSS claims a categorical exclusion from the environmental assessment requirements of 21 CFR 25.20 for approval of *[^{11}C]ER176 Injection* on the basis that the estimated concentration of [^{11}C]ER176 at the point of entry into the aquatic environment will be below 1 part per billion. Additionally, no extraordinary circumstances exist.

13. LIST OF DOCUMENTS

Document 1 Chemistry, Manufacturing and Controls

Document 2 Standard Operating Procedures

Document 3 Master Batch Record

Document 4 Quality Control Record

Document 5 Post-Release Test Record

Document 6 Preparation of ER176 Standard Solution and HPLC Calibration Curve

Document 7 Precursor and Reference Standard Acceptance Form

Document 8 Certificates of Analysis

Document 9 Validation Record

[^{11}C]ER176 Injection: Standard Operating Procedures

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List of Standard Operating Procedures and Supplementary Documents for *[^{11}C]ER176 Injection*

Document Title	Document Number
Preparation of HPLC Mobile Phases	SOP #GP101
Cleaning Procedures for Radiosynthesis Apparatus	SOP #GP102
Cleaning Procedure for Radiochemistry Glassware	SOP #GP103
Preparation of Iodine Column	SOP #GP104
Production of *[^{11}C]ER176 Injection* Part 1: Pre-Synthesis Procedures	SOP #MP201
Production of *[^{11}C]ER176 Injection* Part 2: Synthesis and Formulation	SOP #MP202
Release of *[^{11}C]ER176 Injection*	SOP #QA301
Sampling and Quality Control Procedures for *[^{11}C]ER176 Injection*	SOP #QA302
Analysis of Organic Residues by Gas Chromatography	SOP #QA303
Analytical HPLC Quality Control Method	SOP #QA304
Preparation of ER176 Standard Solution and HPLC Calibration Curve	SOP #QA305
ER176 Precursor and Reference Standard Acceptance Criteria	SOP #QA306
Materials, Instruments and Equipment	Appendix A
Calculations Worksheet	Appendix B
Representative HPLC Chromatograms	Appendix C
Representative Characterization Data for Precursor and Reference ER176	Appendix D
Synthia Program Time Lists	Appendix E

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SOP #GP101

Preparation of HPLC Mobile Phases

Approved by: V.W. Pike Initials: VWP Date: 08/30/12

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

Purpose: To prepare HPLC mobile phases required for purification and quality control analysis of *[^{11}C]ER176 Injection*

Procedure:

1. Preparation of 1 L preparative HPLC mobile phase (1 mM aqueous ammonium hydroxide). Quantities may be scaled as needed.
   1.1. In a 1-L graduated cylinder, add 1 L HPLC grade water.
   1.2. Using a 1-mL syringe, add 1.0 mL of 1 M ammonium hydroxide to the water.
   1.3. Add a clean magnetic stir bar to the graduated cylinder and stir until thoroughly mixed.
   1.4. Vacuum filter the mobile phase through a 0.45 µm nylon filter.
   1.5. Transfer the filtered mobile phase into a clean HPLC reservoir bottle. Label the bottle with a description of contents, date of preparation and expiration date. The mobile phase may be used for two weeks after date of preparation, provided that when not in use it is tightly capped and stored at room temperature.

2. Preparation of 1 L analytical HPLC mobile phase (74% methanol: 26% water). Quantities may be scaled as needed.
   2.1. In a 1-L graduated cylinder, add 260 mL HPLC grade water.
   2.2. Add HPLC grade methanol to the 1-L mark.
   2.3. Add a clean magnetic stir bar to the graduated cylinder and stir until thoroughly mixed.
   2.4. Vacuum filter through a 0.45 µm nylon filter.
   2.5. Transfer the filtered solvent into a clean HPLC reservoir bottle. Label the bottle with a description of contents, date of preparation and expiration date. The mobile phase may be used for two weeks after date of preparation, provided that when not in use it is tightly capped and stored at room temperature.

3. HPLC grade acetonitrile directly from the supplier's bottle may be used without filtration in the preparative system provided that the mobile phase line is fitted with an inlet filter.

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SOP #GP102

Cleaning Procedures for Radiosynthesis Apparatus

Approved by: V. W. Pike Initials: VWP Date: 08/30/12
Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: To clean the Synthia radiosynthesis apparatus and formulation system

Procedure:

1. Pre-synthesis
   1.1. Place a 10-mL round bottom vial in position F31 of the Synthia apparatus and fill with 70/30 ethanol/water.
   1.2. Fill the Synthia syringe reservoir with HPLC grade water.
   1.3. Clean the formulation system transfer lines
      1.3.1. The transfer line between the rotary evaporator and the Omnifit holding column is cleaned by switching manual control valve V2 **ON**, flushing through with 20 mL (min volume) of USP grade absolute alcohol and drying with a constant stream of air.
      1.3.2. The transfer line between the Omnifit holding column and the dose vial is cleaned by switching manual control valve V2 **OFF**, flushing through with 20 mL of USP grade absolute alcohol and drying with a constant stream of air.
   1.4. Clean the formulation addition line by pushing 20 mL (min volume) of USP grade absolute alcohol through the line and dry it by repeatedly flushing air from the syringe through the line.
   1.5. Clean the collection line
      1.5.1. Run clean HPLC mobile phase through the prep HPLC system at 6 mL/min.
      1.5.2. Open manual collection valve V5 to flush at least 5 mL of mobile phase through the collection line. Close V5 when flush is complete. Do not leave V5 open longer than 5 min.
      1.5.3. Disconnect the collection line at the union, manually push through 20 mL (min volume) of USP grade absolute alcohol, dry by repeatedly flushing air from the syringe through the line and reconnect the line
2. Post synthesis
   2.1. HPLC columns
      2.1.1. Wash the analytical HPLC column with 90% acetonitrile/10% water for a minimum of 20 column volumes.
      2.1.2. Place a mixture of acetonitrile/water (at least 50% acetonitrile) in the A1 pump reservoir and ensure there is sufficient acetonitrile in the B1 reservoir. Run HPLC method "ER176

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Wash". Column will be washed with a gradient of 60-100% B and sustained at 100% B for more than 20 column volumes.

2.2. Synthia Apparatus (should be performed when residual radioactivity is below detectable levels).

2.2.1. Dispose of all sharps, disposable vials and other waste from production

2.2.2. Fill a syringe with at least 10 mL HPLC grade water and, using the specialized Synthia injection port needle, flush water through prep injection port to remove any residual KOH.

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SOP # GP103

Cleaning Procedure for Radiochemistry Glassware

Approved by: V. W. Pike Initials: VWP Date: 08/30/12

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

Purpose: To clean production glassware

Procedure:

1. Rinse each item with water, acetone, and/or other appropriate organic solvent as required to remove residue.
   1.1. Synthia glassware: Bathe the glassware in an aqueous 2% solution of Liqui-Nox (Valconox). Bring the solution to a low boil for 5–10 minutes minimum. Allow to cool.
   1.2. Rotary evaporator flasks: Fill with 2% Liqui-Nox solution and scrub gently but thoroughly.
2. Rinse all glassware at least 3 times with deionized water.
3. Carefully check each item. Repeat cleaning steps if required. Gentle scrubbing may be employed.
4. Place glassware in an oven at 70 °C (minimum) and allow to dry completely.

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SOP #GP104

Preparation of Iodine Column

Approved by: V. W. Pike Initials: V WP Date: 08/30/12
Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: To prepare an Iodine Column for the production of [^{11}C]Iodomethane.

Procedure:

1. Packing of the column begins with quartz wool plug Q1, followed by the dry ascarite plug and then the quartz wool plug Q2. This provides the support required for the iodine plug. The column is then completed by packing the rest of the plugs in the following order: quartz wool plug Q3, wetted ascarite and finally quartz wool plug Q4.
2. All quartz wool and ascarite plugs should be packed so that they are firmly in place but not overly compressed
3. Mark out a clean and dry 50 cm glass column as depicted in the diagram below using a permanent marker.
4. Insert enough quartz wool to fill the allocated space for **Q1 plug** (2 cm).
5. Add dry ascarite and fill up to the allocated mark (4 cm).
6. Insert a firmly packed quartz wool plug (**Q2**) at the end of the column.
7. Flip the column up-side-down and add Iodine to the required mark (6 cm).
8. Insert a firmly packed quartz wool plug (**Q3,** 2 cm) to fit within the allocated area.
9. Add sufficient water (one to two drops) to approximately 10 oz ascarite to slightly wet the material. Carefully add the wetted ascarite into the column to the required mark (6 cm) making sure the ascarite is firmly packed.
10. Insert a firmly packed quartz wool plug (**Q4**) at the end of the column
11. Finally, visually inspect the column making sure all materials are within their allocated areas and the ends of the column are free of quartz wool fibers.

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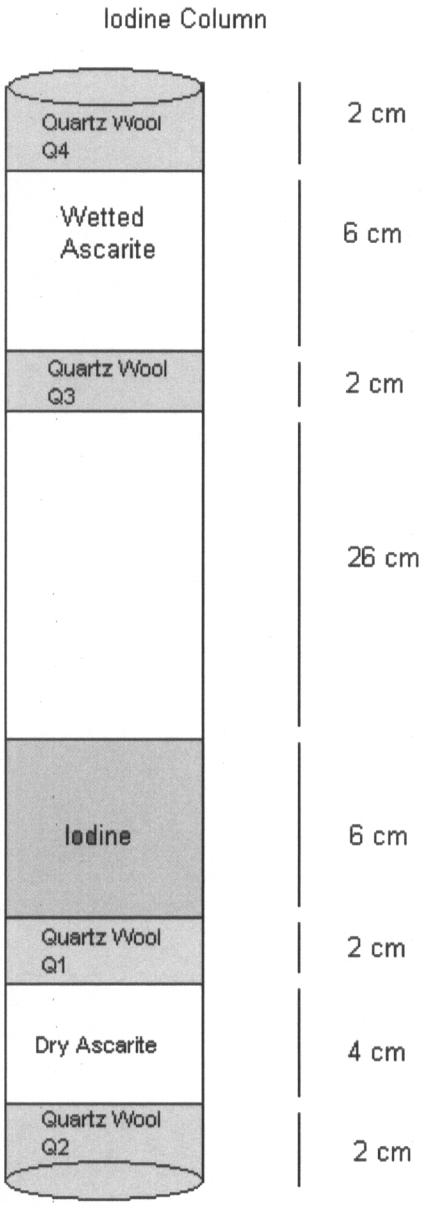
Figure 1: Diagram of packed Iodine Column



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SOP #MP201

Production of *[^{11}C]ER176 Injection* Part 1: Pre-Synthesis Procedures

Approved by: V. W Pike Initials: VWP Date: 08/30/12

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

Purpose: To set up equipment and materials for production of *[^{11}C]ER176 Injection*

Procedure:

1. Ensure the Iodine column has not been used more than five times.
2. Ensure the power is on to all peripheral devices.
3. If needed, turn target inlet valve to the "out" position and request a target flush from a cyclotron engineer, if required. Turn valve back to "in" position when target flush is complete.
4. Confirm that the Valco six-way valve is set to position 3 and the three-way valve is set to "Cryotrap" position.
5. Run a Prep sequence while monitoring RMA, RMB and RMC. Check flow rates and make adjustments to flow meters if necessary.

Rotameter	Flow (scale division)
RMA, recirculation, He	50
RMB, He	50
RMC, H_2	55

6. Connect a flow meter to the [^{11}C]Iodomethane trapping station, position 2.
7. Run Leak Test 1 on GE Microlab
   7.1. Flip Valve 6 (Hot Cell 4) to **ON** position and measure flow rate on flow meter. Flow rate reading should be no less than 15 mL/min.
   7.2. Flip Valve 6 (Hot Cell 4) to **OFF** position and check that the system is leak tight (RMB flow meter displays 0 in less than 3 mins).
   7.3. Cycle through Leak Tests 2 – 6 to complete the Leak Test.
8. Verify the ionization chamber by measuring the ^{57}Co and ^{137}Cs standards. Compare the measurements to the expected measurements for the current 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.
9. 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.

10. Install two 2" 21G needles (vent and [^{11}C]Iodomethane transfer needles) to position 2 of the trapping station.
11. Clean the formulation and collection lines according to SOP# GP102.
12. Reagent Setup
    12.1. Set up all vials and reagents in Table 1 using the diagram in Figure 2 for guidance.[1]

Figure 2: Diagram of Synthia Deck Layout

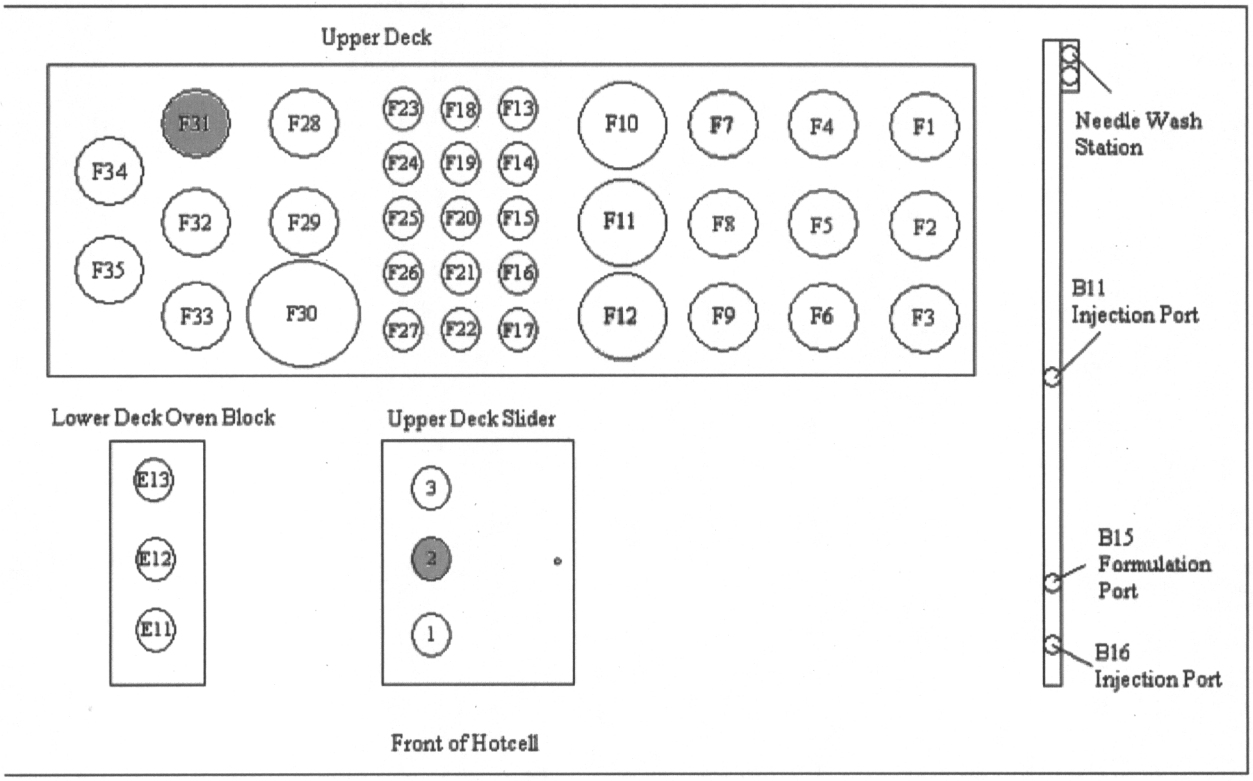

Table 1: Reagent Setup

Position	Tube Type	Solvent/ Material
F31	10-mL round bottom	70:30 Absolute ethanol: HPLC grade water
Slider Position 2	Reaction Vial	Precursor, KOH, DMSO

    12.2. Weigh 0.6 ± 0.06 mg precursor in a tared, high recovery auto-sampler vial.
    12.3. Weigh 1.2 ± 0.2 mg of potassium hydroxide in a tared, high recovery auto-sampler vial.
13. Empty the HPLC waste and the waste container under the needle wash.

[1] 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.

14. Empty and refill the syringe reservoir with HPLC grade water if needed.
15. Add dry ice-ethanol slurry to rotary evaporator condenser and vacuum pump trap.
16. Prepare the formulation vehicle.
    16.1. Add 0.5 mL dehydrated alcohol for injection (USP) to a 10-mL vial of saline for injection. Mix well.
    16.2. Draw up 10.0 mL of the formulation vehicle in a 10-mL syringe and attach syringe to the end of the external addition line into the rotary evaporator.
17. Make sure power is on to the Synthia AutoRad hardware, the oven control box, and the Synthia computer.
18. Start the program "AutoRad.exe" on the Synthia computer. Login in and select 'Load Recipe'. Select the recipe "**ER176 IND**" (refer to Appendix E) and start the recipe.
    18.1. The Synthia recipe will prompt the operator to enter a Reaction Temperature (°C) and Time (seconds). Enter the following: Reaction Temperature 80 °C; Reaction Time 300 secs
    18.2. Follow the recipe instructions until reaching the message "Place the precursor vial in slider position 2, and press OK!".
19. Preparation of the sterile dose vial
    19.1. 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.
    19.2. Prepare the laminar flow hood for operations by turning on if necessary and spraying the interior with 70% aqueous isopropanol.
    19.3. Transfer the following materials to the laminar flow hood. Re-spray the interior and contents of the laminar flow hood with 70% aqueous isopropanol and allow to dry.

Pre-weighed sterile vial 10 mL	4 mm sterile Millex-GV filter
25 mm sterile Millex-MP filter	Two 2", 21G sterile needles
1.5", 20 G sterile needle	Sterile alcohol wipe
1 mL sterile syringe	

    19.4. Assemble the dose vial as depicted in Figure 3 using aseptic technique. Wipe the top of the dose vial with an alcohol wipe and allow to dry prior to assembly.

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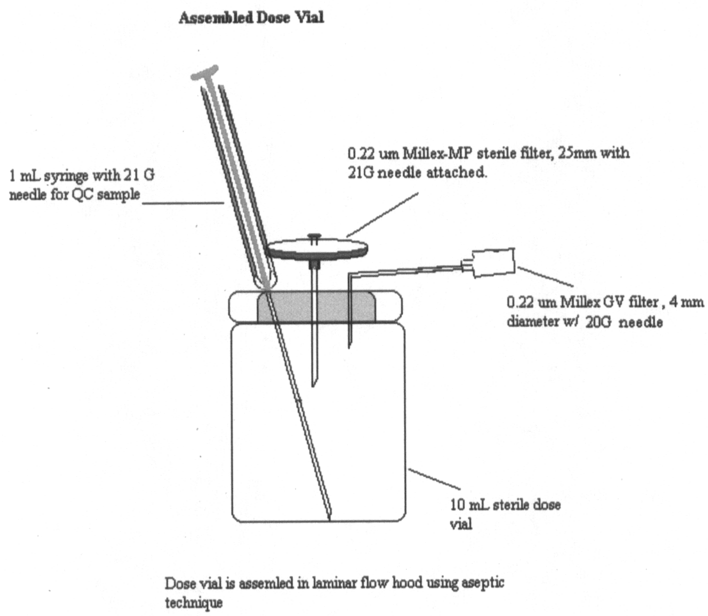
Figure 3: Assembly of Sterile Dose Vial



19.5. Install the dose vial to the formulation line once cleaning of formulation lines is complete.

20. HPLC Setup

20.1. Preparative system

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

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

20.1.3. 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 approximately 3700 psi at 8 mL/min when fully equilibrated. The flow rate may be lowered once the column is equilibrated.

20.2. Analytical

20.2.1. 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'.

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

20.2.3. Equilibrate the column for a minimum of 10 column volumes at initial conditions (refer to Table 2). The initial pressure should be approximately 2400 psi at 2.5 mL/min when fully equilibrated. The flow rate may be lowered once the column is equilibrated.

20.3. System suitability for the analytical HPLC

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20.3.1. Inject ER176 standard (204 ng typical). Refer to SOP # QA304, Analytical HPLC Quality Control Method, for acceptance of the standard injection.

20.3.2. 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 or 118
Detector	Beckman 166	Beckman 166 or 168
Column	Xterra RP-18, 7.8 x 300 mm, 10 µm	Luna C-18, 10 µm, 4.6 x 250 mm
Flow Rate	8 mL/min	2.5 mL/min
Typical initial pressure	3700psi	2400psi
Gradient/ Isocratic	Isocratic	Isocratic
Mobile Phase	37% MeCN / 63% 1 mM ammonium hydroxide (aq)	74% MeOH / 26% water
UV Wavelength	235 nm	235 nm
Bioscan Setting	Diode, 2M	PMT, 20M
Method Name	ER176 Prep	ER176 Analytical
Trigger	External	Manual
Sample Loop Size	5 mL	2 ml

21. Prepare autosampler vial for GC analysis

21.1. Place a conical insert into an Aglient autosampler vial.

21.2. Add 50 µL of 386 ppm propionitrile standard to the vial and label vial with "ER176 GC" and the date.

21.3. Load GC method "ISPRCN.m" at least 30 minutes before the expected analysis time and check system after equilibration period to ensure software status is "Ready".

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SOP #MP202
Production of [^{11}C]ER176 Injection Part 2: Synthesis and Formulation

Approved by: V. W. Pike Initials: VWP Date: 08/30/12
Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Purpose: To synthesize and purify [^{11}C]ER176 and formulate for injection.

Procedure:

1. [^{11}C]Iodomethane Synthesis and Trapping
   1.1. Upon end of bombardment and verbal confirmation from a cyclotron engineer, open the [^{11}C]carbon dioxide valve to hot cell 3 and press run button on the GE MeI MicroLab
   1.2. Once transfer has been initiated, prepare the precursor solution by adding 0.4 mL anhydrous DMSO to the vial containing 0.6 ± 0.06 mg ER176 precursor. Mix well to dissolve and transfer the solution to the vial containing 1.2 ± 0.2 mg KOH. Mix well, crimp seal and place the vial in Synthia trapping station 2. Proceed with recipe until reaching the message "Press OK when C-11 MeI begins to release."
   1.3. When Step 7 "Methane waste" on the GE MeI MicroLab ends (about 12 min after EOB), turn on valve 6 to transfer radioactivity to Hot cell 4 and hit "OK" on the Synthia program to begin trapping.
   1.4. When the radioactivity is maximized in the reaction vial (about 2-3 min after initiation of [^{11}C]iodomethane trapping), turn off valve 6 and follow the Synthia instructions to continue.
2. Reaction and Purification
   2.1. The reaction vial will be moved by robotic arm (Gilson ASPEC Liquid Handler) into an oven and will be heated at 80°C for 5 mins.
   2.2. During (or prior to) the 5 min reaction, the preparative HPLC should be made ready for injection (HPLC method "ER176 Prep.met"). Set up a single run, ensure that the flow rate is 8 mL/min and the pressure is within the expected range, and that the message "waiting for trigger" appears at the bottom of the HPLC screen.
   2.3. The reaction mixture will be diluted and loaded into the HPLC injector loop by the robotic arm and the HPLC data collection will be triggered automatically.
   2.4. Turn on the vacuum pump and raise the water bath up to the flask before the peak is expected to elute.
   2.5. Collect the product peak by switching manual control valve V5 **ON**. Refer to Appendix C for a sample preparative trace. Switch off manual control valve V5 once collection is complete.

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2.6. Concentrate the product to dryness under reduced pressure. Typical evaporation time is 1.5–2 minutes.

2.7. Turn off the vacuum pump and vent the rotary evaporator flask through the formulation line.

3. Formulation

3.1. Add formulation vehicle to the rotovap flask through the formulation line while spinning the flask.

3.2. Open manual valve V2 and pull the reconstituted product solution into the Omnifit holding column.

3.3. Close manual valve V2 and push the product solution into the sterile dose vial. Visually verify that the formulated [^{11}C]ER176 solution has been completely transferred to the dose vial.

4. Completion

4.1. Complete all information required on the Master Batch Record.

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

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SOP #QA301

Release of *[^{11}C]ER176 Injection*

Approved by: V. W. Pike Initials: VWP Date: 08/30/12

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

Purpose: To qualify *[^{11}C]ER176 Injection* for release and fully describe release and post-release test criteria.

Procedure:

1. Release Tests – the following must be completed and all acceptance criteria met **before** release from the PRSS/MIB/NIMH production site. Refer to SOP #QA302 for detailed procedures.

Test	Acceptance Criteria	Test Description
Appearance	Formulated dose is clear, colorless and free of particulates	Visual inspection
Bacterial endotoxins	EndoSafe®-PTS *Limulus* amebocyte lysate (LAL) test	Amount of endotoxin not to exceed 175 EU in injected volume
Chemical purity	Maximum volume contains NMT[2] 10 µg of carrier and NMT 1.0 µg ER176 equivalent impurity	HPLC QC procedure
Membrane filter integrity	No air passage through filter observed at 45 psi	Pressurize sterile filter above 45 psi to determine whether membrane has remained intact
pH	pH 4.0-7.5	Measured using narrow range pH paper
Radiochemical identity	Retention time within ± 1.0 min of a standard injection of ER176	HPLC QC procedure
Radiochemical purity	NLT[3] 95% [^{11}C]ER176	HPLC QC procedure
Radionuclidic identity	The measured half-life is 20.4 ± 2 min	Calculated half life from two measurements at least 3 minutes apart
Residual solvents	≤ 4.1 mg acetonitrile in the injectable dose[4] and ≤ $1x10^5$ ng/µL ethanol	Gas chromatography with flame ionization detection
Specific radioactivity	NLT 500 mCi/µmol at EOS	HPLC QC procedure

[2] Not More Than
[3] Not Less Than
[4] Refer to USP <467>

2. Labeling and Release

2.1. If the product meets all acceptance criteria for the above tests, complete two of the following labels:

[^{11}C]ER176 Injection

Sterile, apyrogenic saline solution for intravenous administration

Caution: New drug limited by Federal law to investigational use only

Expires 1 h after calibration Half-life of ^{11}C is 20.4 min

Concentration:_____mCi/mL Time:_____

Activity:_____mCi Volume:_____

Date:_____ Lot #:_____

Attach one label to the product vial and one to the Post-Release Test Record. Activity and concentration measurements are calibrated to the EOS (End of Synthesis) time.

2.2. Complete the Quality Control Record and sign and date to authorize release from the production/ quality control area. The dose vial containing the product may then be transported to the PET scanning center.

3. Post-Release Tests

The following test must be completed and acceptance criteria met within the time frame specified below. Refer to SOP #QA302 for detailed procedures.

Test	Test Description	Acceptance Criteria
Sterility	NIH Clinical Center Microbiology Lab	No aerobic or anaerobic growth observed

3.1. A sample for sterility testing should be submitted to the NIH Laboratory Medicine, Microbiology Department when the radioactivity in the sample is below the level of detection of a pancake GM detector; typically 24 h. Sampling and submission may be delayed when facilities are closed or personnel unavailable but should be completed as soon as possible.

3.2. Test results should be reported on the Post-Release Test Record and the form signed and dated when complete. Sterility result form received back from Microbiology should be filed with the Post-Release Test Record.

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4. Batch Record and Data Filing

   The Batch Records and all supporting data should be filed according to current PRSS/MIB/NIMH practices. Typically, all original paperwork for a batch is attached together and filed in a clearly labeled binder in the PRSS facility. Electronic data filing may be implemented in the future. The complete batch record should include the following:

   Master Batch Record
   Quality Control Record
   Post-Release Test Record
   Semi-Preparative HPLC report
   Analytical HPLC reference standard injection report
   Analytical HPLC [^{11}C]ER176 Injection QC injection report
   Residual solvent analysis / gas chromatography report
   Calculations worksheet
   Sterility report
   Any ancillary data obtained during the production or memos detailing any deviations

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SOP #QA302

Sampling and Quality Control Procedures for *[^{11}C]ER176 Injection*

Approved by: V. W. Pike Initials: VWP Date: 08/30/12

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

Purpose: To fully describe all pre- and post-release quality control testing required for *[^{11}C]ER176 Injection*.

Procedure:

1. Sampling of the *[^{11}C]ER176 Injection* for quality control.
   - 1.1. Use the sterile sampling syringe, previously inserted into the dose vial under aseptic conditions, to remove a 600 µL (minimum) sample.
   - 1.2. Dispense 300 µL (minimum) to a clean vial for HPLC analysis
   - 1.3. Dispense 300 µL (minimum) to a clean, pyrogen-free vial or test tube.
2. Release Tests – these tests must be completed prior to release of the *[^{11}C]ER176 Injection* from the production facility and all resultant data recorded on the Quality Control Record.
   - 2.1. Volume – weigh the dose vial after the QC sample has been removed. Record the mass and calculate the volume using the previously recorded empty vial mass, assuming a density of 1 g/mL. There is no specific release criteria for volume, but the expected value is typically 8-8.5 mL.
   - 2.2. Yield - Measure the activity in the dose vial after the QC sample has been removed. The total activity must be greater than 20 mCi. The time and activity of this measurement are recorded on the Master Batch Record.
   - 2.3. pH - Dispense 1-3 drops of *[^{11}C]ER176 Injection* on to a strip of narrow-range pH paper. The indicated result should be between 4.0 and 7.5.
   - 2.4. Membrane Filter Integrity
     - 2.4.1. Remove the intact filter assembly (filter and needle) from the sterile dose vial and submerge the tip of the needle in water contained in a transparent vessel.
     - 2.4.2. With the compressed air valve closed, attach the air line outside the hot cell to the product formulation line. Ensure the dial is lowered so that the initial pressure will be less than 20 psi and open the air valve.
     - 2.4.3. Slowly increase the pressure to 45 psi and observe the needle outlet. Hold pressure at 45 psi for 10 seconds If no bubbles are observed at 45 psi, the filter passes the integrity test.
     - 2.4.4. Lower the pressure to below 20 psi and close the compressed air valve.
   - 2.5. Appearance - Visually inspect the contents of the dose vial. The product should be a clear, colorless liquid free of particulates or cloudiness.
   - 2.6. HPLC Analysis and Resulting Calculations
     - 2.6.1. Using a Hamilton syringe (or equivalent) fitted with a blunt tip for HPLC injection, remove exactly 100 µL *[^{11}C]ER176 Injection* from the quality control vial (Step 1.2.).

2.6.2. Measure the background radiation in the ionization chamber by immersing the empty dipper in the ionization chamber.

2.6.3. Measure the syringe containing 100µL *[^{11}C]ER176 Injection* in the ionization chamber. The time of this measurement is recorded as the End of Synthesis (EOS) time.

2.6.4. Inject the sample on the analytical HPLC system and analyze according to the procedures in SOP #QA304: Analytical HPLC Quality Control Method.

2.6.5. Calculate the net injected activity as the difference between the 100 µL measurement and the background. Both values should be entered into the Calculations Worksheet.[5]

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

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

$$\%\text{Purity} = \frac{\mu\text{g ER176 carrier}}{\mu\text{g carrier} + \mu\text{g impurities}} \text{x}100 \qquad \textbf{Eq. 2}$$

The mass of carrier and carrier-equivalent impurities are calculated by the equation:

$$\mu g = \frac{peak\ \ area}{m} \times MW \qquad \textbf{Eq. 3}$$

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

2.6.7. Radiochemical Purity is determined from the HPLC Bioscan by the equation:

$$\%Purity = \frac{\text{Product Peak Area}}{\text{Total Peak Area}} \times 100 \qquad \textbf{Eq. 4}$$

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

$$Concentration = \frac{\text{mCi}_{(\text{Net})}}{100\ \mu L} \times \frac{1000\ \mu L}{1\,\text{mL}} \qquad \textbf{Eq. 5}$$

2.6.9. Chemical and Radiochemical Identity is confirmed by comparing the HPLC 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.

2.6.10. 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:

[5] A copy of the Calculations Worksheet may be found in Appendix B

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$$\mu\text{mol carrier} = \frac{\text{peak area carrier}}{\text{m}} \qquad \textbf{Eq. 6}$$

Where the slope is in units of area x μmol^{-1} and the area is from the UV trace at 235 nm. Specific Activity is calculated by:

$$s.a. = \frac{\text{mCi}_{(\text{Net})}}{\mu\text{mol Carrier}} \qquad \textbf{Eq. 7}$$

2.7. Radionuclidic Identity is determined from the experimentally determined half life calculated from two measurements taken at least 3 minutes apart.

$$t_{1/2} = \frac{\ln(2) \times \Delta t}{\ln\left(\frac{A_0}{A}\right)} \qquad \textbf{Eq. 8}$$

Where A_0 is the first radioactivity measurement, A is the second activity measurement, $t_{1/2}$ is 20.4 minutes, and **Δt** is the difference in time in units of minutes.

2.8. Residual Solvent

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

2.8.2. Transfer 50 μL of *[^{11}C]ER176 Injection* from the pyrogen-free vial into a prepared autosampler vial containing 50 μL of the calibrated propionitrile standard. Make sure that no air bubbles are present.

2.8.3. Load the prepared sample into the autosampler tray. Click the "Start" button.

2.8.4. Data acquisition may be stopped after 3.5 min; chromatogram will be automatically analyzed and the report will print results in units of ng/μL.

2.8.5. The sample must contain no more than 1 x 10^5 ng/μL of ethanol (EtOH, t_R *ca.* 2.27 min) and no more than 4.1 mg of acetonitrile (MeCN, t_R *ca.* 2.67 min.) in the maximum allowable injected volume (equivalent to 410 ng/μL for a 10 mL dose).

2.9. Bacterial Endotoxins

Note: Steps 2.9.1.-2.9.3. may be performed before the [^{11}C]ER176 Injection *synthesis is complete*

2.9.1. Open the top of the EndoSafe unit and press the "Menu" key. Allow the unit to go through all of its self tests.

2.9.2. Remove a test cartridge from storage and allow to come to room temperature. For the first use of a new cartridge lot, the certificate of analysis should be filed in the EndoSafe binder and the unit will prompt for the calibration code during setup.

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2.9.3. When the unit is ready it will prompt the operator as follows

PROMPT: **Insert Cartridge**	Insert the cartridge fully.
PROMPT: **Enter OID**	Enter an operator ID.
PROMPT: **Lot #**	Enter the lot number of the cartridge.
PROMPT: **CANCEL or ENTER**	Press "Enter" to accept the lot number.
PROMPT: **Sample Lot#**	Enter the sample lot number (yymmddxx).
PROMPT: **Sample ID**	Enter the sample ID (ER176)
PROMPT: **Dilution**	Enter the dilution factor (20)
PROMPT: **Add sample and press enter**	Load sample as follows when ready.

2.9.4. Transfer 50 µL of *[^{11}C]ER176 Injection* from the pyrogen-free vial to a clean pyrogen-free tube. Dilute with 950 µL of sterile water. Load 25 µL of the diluted sample into each of the four wells on the EndoSafe plate and press the "Enter" key.

2.9.5. When the test is complete (13.5 min) a tone will sound. The display will cycle through the results.

Sample	The mean detected value for the level of endotoxin present in the sample. A 'less than' symbol will appear before the value if it is below the detection limit of the unit (2.0 EU/mL for a 20:1 dilution).
Sample %CV	The coefficient of variation (CV) for the duplicate sample channels. Must be less than or equal to 25% for a valid test.
Spike	The mean detected value for the level of endotoxin present in the spike channels. The true value is found on the certificate of analysis for the cartridge lot.
Spike %CV	The CV for the duplicate spike channels. Must be less than or equal to 25% for a valid test.
Recovery	The percent recovery of spike in the spike channels. Must be between 50% and 200% for a valid test.

2.9.6. Record the test results on the Quality Control Record and ensure all criteria have been met. The FDA endotoxin limit for drug products is 5 EU (endotoxin units) per kg of subject body mass. The PRSS/MIB/NIMH facility produces doses for adult subjects only, and as such the subject body mass is anticipated to be greater than 35 kg. Accordingly, the endotoxin limit is set to no more than 175 EU in the maximum injectable dose. In the event that a subject weighs less than 35 kg, the limit may be adjusted accordingly and noted on the Quality Control Record.

3. Post-Release Test - Sterility

Note: Sample should be submitted as soon as the radioactivity in the sample is below the level of detection of a pancake GM detector; typically 24h. In the event that [^{11}C]ER176 Injection *is produced prior to a weekend or any other extended period during which the Federal Government is closed, sampling and submission may be delayed until facilities reopen and personnel is available but should be completed as soon as possible at that time.*

3.1. Confirm the absence of radioactivity in the sample using a pancake GM detector.
3.2. Prepare the laminar flow hood for aseptic use.
3.3. Using a permanent marker, record the lot number and date of submission on one Bactec aerobic vial and one Bactec anaerobic vial. **Do not write on barcodes of the Bactec vials.**
3.4. Place the two Bactec vials, the product dose vial, two aseptic alcohol wipes and a sterile syringe with needle in the laminar flow hood. Spray the items with 70% isopropanol and allow to dry before proceeding.
3.5. Using aseptic technique, remove the flip-caps of the Bactec vials and wipe the tops of the vials and the product dose vial. Allow to dry.
3.6. Remove 200 µL from the product dose vial and add 100 µL to each of the Bactec vials.
3.7. Fill out the "Request for Sterility Test" form and submit the sample and form to the NIH Clinical Center Microbiology Lab. Retain a copy of the submission form with the Batch Record until results are returned in approximately 2-4 weeks.
3.8. When results are returned, indicate result on Post-Release Test Record and file with Batch Record.
3.9. *In Case of Positive Result.* If growth is reported: a) notify the Principal Investigator; b) ask for the identity of the microorganism from the microbiology lab and; c) file a report on the investigation and follow-up results in the GMP investigations file.

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

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SOP #QA303

Analysis of Organic Residues by Gas Chromatography

Approved by: V.W.Pike Initials: VWP Date: 08/30/12

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

Purpose: To test for residual volatile solvents in *[^{11}C]ER176 Injection.*

Procedure:

1. Operation and Analysis
   - 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' and enter the correct sample information.
   - 1.7. Add equal amounts of *[^{11}C]ER176 Injection* (test sample) and the calibrated propionitrile standard (50 µL 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 3.5 minutes. The sample must contain no more than 1 x 10^5 ng/µL of ethanol (EtOH, t_R *ca.* 2.27 min) and no more than 410 ng/µL of acetonitrile (MeCN, t_R *ca.* 2.67 min.) in the maximum allowable injected volume (equivalent to 4.1 mg for a 10 mL dose).
   - 1.9. Include a copy of the GC report with the Batch Record.
2. Post-run operations
   - 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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Materials and Equipment:

Agilent 6850 Gas Chromatograph 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)
Parker Balston H2-90 Hydrogen Generator
Inlet liner: split inlet glass liner with glass wool packing (Agilent part number, 5183-469119251-60540)
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);
Autosampler conical glass insert (Agilent part no. 5183-2085)
Acquisition and data processing software: GC Chem Station (version: Rev. A.09.03 [1417])
In-house air purified by Parker Balston Zero Air Generator, Model 75-83NA

Method "ISPRCN.M" Parameters

<table>
<tr><td colspan="3">Injection port:
split sample injection
split ratio of 20: 1 T = 250 °C</td><td>Carrier gas:
Helium 2 mL/min</td></tr>
<tr><td colspan="3">Column temperature gradient:
<u>Time</u> <u>Temperature</u> <u>Duration</u>
t_0 T=50 ° C 1 min
$t_{1\ min}$ T = 150 °C 5 min
$t_{6\ min}$ 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</td><td>Detector:
FID 250 °C
H_2 40 mL/min and air at 450 mL/min.
He make-up 45 mL/min. Detector</td></tr>
<tr><td colspan="3">Autosampler:
Syringe size 10 µL
Sample injection volume 1 µL.</td><td>Needle/Syringe wash:
Before injection 4
After injection 2</td></tr>
</table>

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SOP #QA304

Analytical HPLC Quality Control Method

Approved by: V. W. Pike Initials: VWP Date: 08/30/12

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

Purpose: To perform HPLC analysis of [^{11}C]ER176 Injection for quality control.

Procedure:

1. System preparation

1.1. Load method "ER176 Analytical" and download to instrument. Ensure system is prepared according to the parameters below:

Component/Parameter	Requirement/Setting
HPLC Pump	Beckman 118 or 126
HPLC Detector	Beckman 166 or 168
Column	Phenomenex Luna C18, 10 µm, 4.6 x 250 mm
Flow Rate	2.5 mL/min
Typical Pump Pressure	2.4 kpsi
Mobile Phase	Methanol:water, 74:26 v/v
Isocratic/Gradient	Isocratic
UV Wavelength	235 nm
Bioscan Setting	PMT, 20 M for 50-2000 µCi injection, may be adjusted
Instrument Trigger	Manual
Sample Loop Size	2 mL

1.2. Equilibrate the column at initial conditions for a minimum of 20 column volumes. The initial pressure should be approximately 2.4 kpsi at 2.5 mL/min once fully equilibrated. The flow rate may be reduced once column is equilibrated.

2. System Suitability

2.1. Start a single run and inject an accurate volume of a known concentration of ER176 reference standard (typically 204 ng). After the ER176 has eluted (approximately 5.0 min), analyze the chromatogram. The integrated peak area must be within ± 10% of the expected peak area (based on the established calibration curve) to validate the HPLC system. In the event that the standard does not meet the criterion, a new standard solution and/or calibration curve should be made or a new analytical column installed as applicable.

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2.2. Wash the analytical injection port with ethanol and/or acetonitrile and reduce flow rate until needed for quality control analysis of *[^{11}C]ER176 Injection*.

3. Quality control analysis of *[^{11}C]ER176 Injection*.
   3.1. Prepare and measure a sample for injection according to the procedures described in SOP #QA302. Start a single run and inject the sample. Enter the sample and background radioactivity measurements in the calculations worksheet.
   3.2. After the run is complete (typically 8 min), analyze the chromatogram. Record the retention time of the UV peak and the percent area of the Bioscan trace on the Quality Control Record.
   3.3. Enter the carrier peak area and the sum of the areas of any impurity peaks in the calculations worksheet, excluding peaks present in the formulation vehicle blank which arise from the 0.9% sodium chloride for injection, USP. See formulation vehicle blank HPLC chromatogram in Appendix C for representative chromatogram.

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SOP #QA305

Preparation of ER176 Standard Solution and HPLC Calibration Curve

Approved by: V. W. Pike Initials: VWP Date: 08/30/12

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

Purpose: To prepare a standard solution of ER176 and generate a calibration curve to determine the mass of the carrier in *[^{11}C]ER176 Injection*.

Procedure:

1. Prepare a 100 ng/µL (approximate) stock solution of reference ER176.
   1.1. Place a clean 25-mL volumetric flask on an analytical balance and tare the flask.
   1.2. Weigh approximately 2.5 mg of ER176 reference standard into the flask. Record the exact mass on Form #QA305.
   1.3. Dilute to the 25-mL mark with anhydrous acetonitrile. Mix thoroughly.
   1.4. Transfer the contents of the flask to a septum-sealed 30 mL sterile dose vial. Label the vial with "ER176 Stock", exact concentration and date of preparation. Record exact concentration on Form #QA305.
   1.5. Store the vial at or below 4 °C when not in use.
2. Prepare a 2 ng/µL (approximate) solution of reference ER176 for HPLC injection.
   2.1. Transfer precisely 200 µL of the ER176 stock solution to a clean 10-mL volumetric flask.
   2.2. Dilute to the 10-mL mark with anhydrous acetonitrile. Mix thoroughly.
   2.3. Transfer the contents of the flask to a septum-sealed 10-mL sterile dose vial. Label the vial with "ER176 for HPLC", exact concentration and date of preparation. Record exact concentration on Form #QA305.
   2.4. Store the vial below at or below 4 °C when not in use.
3. Prepare a calibration curve on the analytical HPLC system that will be used for quality control analysis of *[^{11}C]ER176 Injection.*
   3.1. Remove the solution for HPLC injection from the freezer and allow to warm to room temperature before use.
   3.2. Remove a 1-mL (approximate) aliquot of the solution into a separate vial; keep capped when not in use.
   3.3. Perform replicate (minimum 5) injections of a minimum of 4 different volumes. The range of volumes should be chosen such that it brackets the expected concentration range of *[^{11}C]ER176 Injection*, approximately 20-200 ng. Perform injections using analysis and method conditions described in SOP #QA304.
   3.4. Calculate the molar mass of each injection volume; the molecular weight of ER176 is 353.85 g/mol.

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3.5. Calculate the mean and percent relative standard deviation (%RSD) of each replicate set. The %RSD must be no more than 3%, and in the event this limit is exceeded, 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 injected mass in units of µmol. Include the point (0,0) (forced origin). Calculate the correlation coefficient (r^2), which must be no less than 0.98. Report the slope in units of **area x µmol^{-1}**.

3.7. Record the data and calculations on Form #QA305. File a copy of this form, the calibration curve plot and all supporting HPLC chromatograms with the IND folder or in a *[^{11}C]ER176 Injection* Supplementary Records Binder, stored with the *[^{11}C]ER176 Injection* batch records.

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SOP #QA306

ER176 Precursor and Reference Acceptance Criteria

Approved by: V. W. Pike Initials: VWP Date: 08/30/12

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

Purpose: To establish the testing and acceptance criteria for ER176 reference and precursor compounds.

1. Overview

The procedure at PRSS/MIB/NIMH for the radiosynthesis of *[^{11}C]ER176 Injection* requires *N*-Desmethyl-ER176 (hereafter referred to as ER176 precursor). Reference ER176 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 *[^{11}C]ER176 Injection*.

If acceptance testing is performed by the supplier of the material and supporting analytical data is provided, testing is not required to be done at the PRSS facility. If any required tests are not performed by the supplier, following are the test methods and acceptance criteria for both compounds. The typical 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 as needed for chemical purity using HPLC analysis and chemical identity using LC-MS analysis.

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2. Acceptance Test Method Parameters/ Sample Preparation

2.1. Material Source

The ER176 precursor and reference ER176 are obtained from an outside source provided that all acceptance criteria contained in this document are met.

2.2. Acceptance Tests[6]

2.2.1. NMR

Sample Prep

Fifteen to twenty milligrams of the material to be tested (either ER176 precursor or ER176 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

2.2.2. LC-MS

Sample Prep

10 µg/mL ER176 precursor in MeCN or MeOH
10 µg/ mL ER176 reference material in MeOH.

Instrument

LCQ Deca LC/MS with Xcalibur V 2.0 software (Thermo Fisher Scientific).

Method Parameters

Column:	Luna C18, 3 µm, 50 x 2 mm (Phenomenex)
Flow Rate:	200 µL/ min
Mobile Phase:	Water-methanol-acetic acid (90: 10: 0.5 by vol.) (A) Methanol-acetic acid (100: 0.5, v/v) (B)
Gradient/ Isocratic:	Gradient
Injection Volume:	2 µL
Ionization:	Electrospray
Detection Range:	*m/z* 150 to 750

2.2.3. HPLC

Sample Prep

10 µg/mL ER176 precursor or ER176 reference material in 50% MeCN (aq).

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

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Instrument

Beckman Coulter 126 pump and 166 or 168 detector with 32 Karat software.
Phenomenex Luna C18 10 µm, 4.6 x 250 mm column.

Method Parameters

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

2.3. 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 235 nm greater than 95%. Refer to reference spectra attached.

3. Documentation

3.1. The Precursor and Reference Acceptance Form should be filled out completely with the appropriate spectra attached. The completed form and attachments should be stored with the IND folder or in a *[^{11}C]ER176 Injection* Supplementary Records Binder and stored with *[^{11}C]ER176 Injection* Batch Records.

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Appendix A: Materials, Instruments and Equipment

This Appendix provides a list of the materials, instruments and equipment to be used in the production and quality control procedures for [^{11}C]ER176 Injection. Substitution with equivalent materials, instruments, and/or equipment is permitted provided that the substitution is documented and approved by the PRSS chief.

Materials

Materials	Manufacturer or Supplier	Catalog/Part #
Water for Injection, sterile	Abraxis Pharmaceutical Products	918510
Water, HPLC grade	EM Science *or*	WX0008-1
	Sigma-Aldrich	270733
Acetonitrile, HPLC grade	Burdick and Jackson *or*	017-4
	Sigma-Aldrich	34821
Methanol, HPLC grade	Fisher Scientific Inc. 47777 Warm Springs Blvd Fremont, CA 94539	A452
Ammonium hydroxide, 1N	Ricca Chemical Company, Arlington, TX 76012	642-16
Analytical HPLC column (Phenomenex Luna 10 µm, 4.6 x 250 mm)	Phenomenex	00G-4253-E0
Semi-prep HPLC column (XTerra RP18 10 µm, 7.8x 300 mm)	Waters	186001166
Dimethyl sulfoxide	Sigma-Aldrich	276855
Potassium Hydroxide	Sigma-Aldrich	306568
ER176 precursor	Dip. Scienze Farmaceutiche-Università di Pisa	ER176_040711
ER176 reference standard	Dip. Scienze Farmaceutiche-Università di Pisa	ER169_040711
Ethanol, absolute	Warner-Graham *or*	6505001050000
	Pharmco-AAPER	111000200, E200
0.9% Sodium chloride for injection, USP	Abraxis Pharmaceutical Products	918610
Dehydrated alcohol for injection, USP	American Regent, Inc.	0517-8571-10
Sterile empty vial, 10 mL	Abbott Laboratories	5618-11
Sterile filter (Millex MP, 0.22 µm; 25 mm)	Millipore	SLMP025S5
Sterile vent filter (Millex GV, 0.22 µm; 4 mm)	Millipore	SLGV004SL
Sterile syringe, 1 mL	Henke Sass Wolf	4010.200V0
Sterile needle, 20G x 1½"	Becton-Dickinson	305176
Sterile needle, 21G x 2"	Becton-Dickinson	305129
Alcohol prep pads	Kendall Healthcare	6818
0.45 micron nylon membrane filter	Phenomenex	AFO-0504
EndoSafe test cartridges	Charles River Laboratories	PTS20F
Sterile pipette tips	Eppendorf	0030 010.035

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Instruments and Equipment

Operation/Function	Manufacturer	Model	Serial #
Radiosynthesis	General Electric	PETtrace Methyl Iodide Micro Lab	27740
Radiosynthesis	Uppsala University	Synthia	Cecilia
HPLC purification	Beckman Coulter	System Gold 126 pump	342-2361
		System Gold 166 detector	322-2189
HPLC quality control	Beckman Coulter	System Gold 126 pump	342-1307
		System Gold 166 detector	312-2185
HPLC radioactivity detection	Bioscan	Flow-Count PIN detector	
		Preparative system	0605-317
		Analytical system	0605-319
Radioactivity measurement	Biodex	AtomLab 300 dose calibrator	01332706
Mass/volume measurement	Acculab	PP-250-B	0492AN025
Mass measurements	Sartorius	CP225D analytical balance	13907271
Verification of analytical balances	Mettler Toledo Inc	ASTM Class 4 weighting standard 1.0 mg	11123607
Verification of portable balances	Sartorius	Class U calibrated weights 10.0 mg	09-970198-1
Measurement of HPLC sample for quality control	Hamilton	Microliter syringe, 100 µL	NA
Bacterial endotoxin test	Charles River Laboratories	EndoSafe	1247 or 1248

Appendix B: Calculations Worksheet

This Appendix provides the worksheet used to perform quality control and analysis calculations. Cells shaded gray are for user input; all other cells are locked and password protected.

Date (mm/dd/yy):				
Batch # (ER176-yymmddxx):	ER176-			
Chemist:		301-451-3928 (lab)		
Radioconcentration (mCi/mL) =	=B18*10	=C18		
Specific Radioactivity (mCi/μmol) =	=B18/B24	=C18		
ER176 Carrier Concentration (μg/mL) =	=B25*10			
Total Volume of Formulated [^{11}C]ER176 for Injection (mL) =				
Maximum Allowable Injection Volume (mL) =	=MIN(B29:B30)			
Enter slope of calibration curve, M (X= μmol, Y= UV peak area):	873106000	90% Expected = 453023		
Date of calibration curve preparation:	8/21/2012	Expected Area = 503359		
		110% Expected = 553695		
Enter radioactivity in whole dose vial (mCi):				
Enter radioactivity in 100 μL aliquot (mCi):		Enter End of Synthesis (EOS) Time →		
Enter background radioactivity before injection (mCi):				
Net activity in 100 μL aliquot (mCi) =	=B16-B17			
Enter UV peak area of ER176 carrier peak:				
Enter sum of areas of all impurity peaks:				
Percent chemical purity of ER176 =	=B19/(B19+B20)*100			
Molecular weight of ER176 =	420.52			
μmol of ER176 in 100 μL aliquot	=B25/B23			
Mass of ER176 in 100 μL aliquot (μg) =	=(B19/B11)*B23			
Mass of ER176 equivalent impurity in 100 μL aliquot (μg) =	=(B20/B11)*B23			
Maximum allowable injected carrier mass (μg):	5			
Maximum allowable injected impurity mass (μg):	0.5			
Maximum allowable injection volume based on carrier mass (mL) =	=B27/(B25*10)	Maximum injection volume is the lesser of the two values when calculated from maximum allowable carrier or maximum allowable impurity		
Maximum allowable injection volume based on impurity mass (mL) =	=B28/(B26*10)			
Second measurement of whole dose vial for half-life calculation (mCi)				
Time between measurements (min)				
Calculated half-life (min)	=(LN(2)*B33)/LN(B15/B32)			

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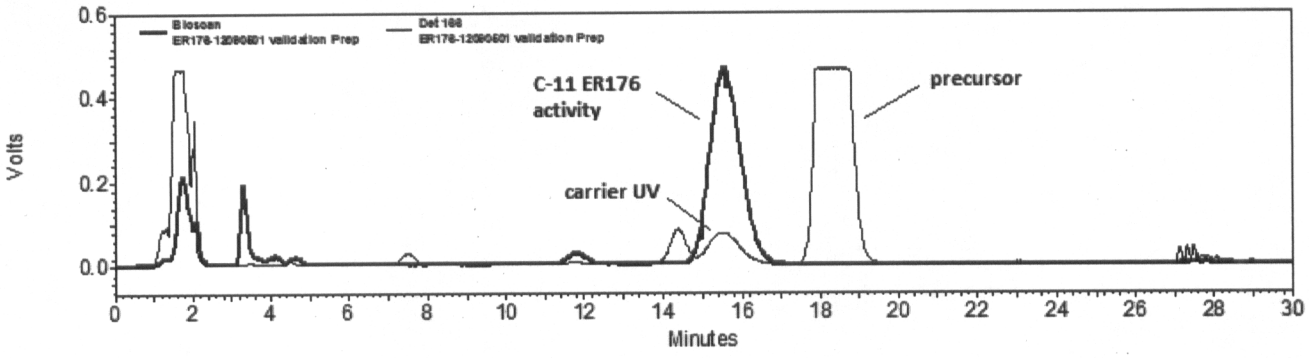
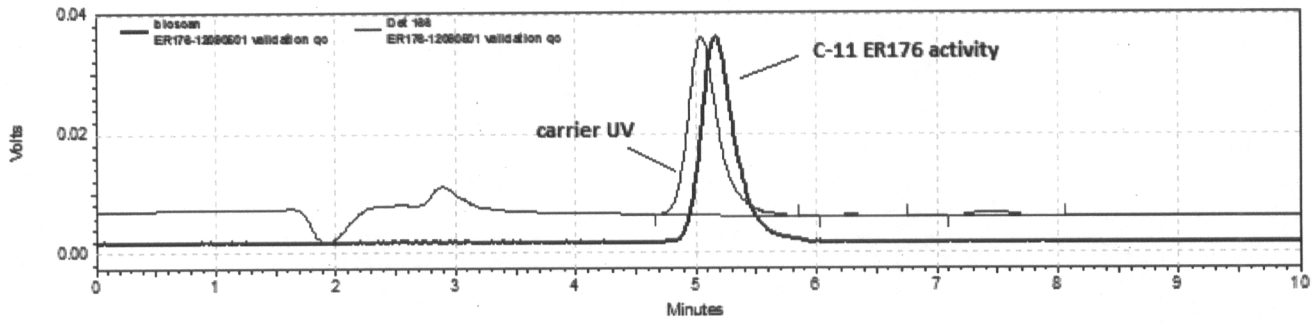
Appendix C: Representative HPLC Chromatograms

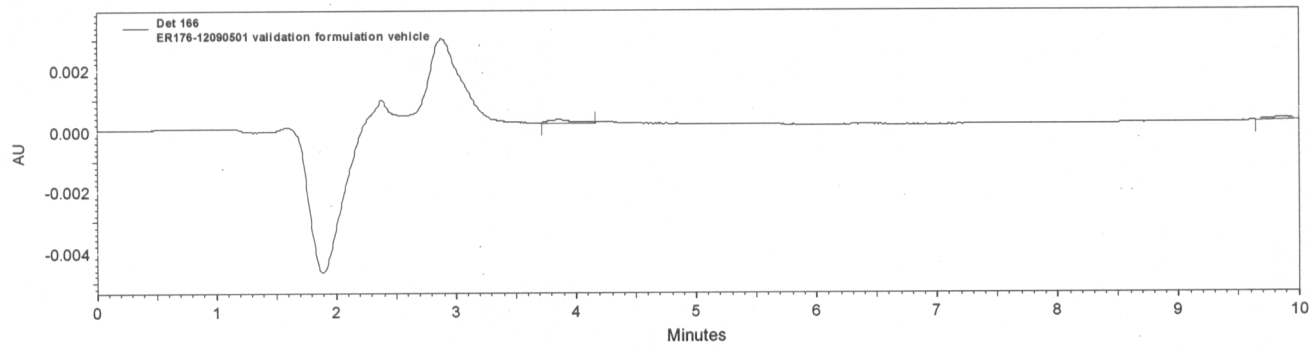
Representative Semi-Preparative HPLC Chromatogram



Representative Analytical HPLC Chromatogram



Representative Analytical HPLC Chromatogram of Formulation Vehicle



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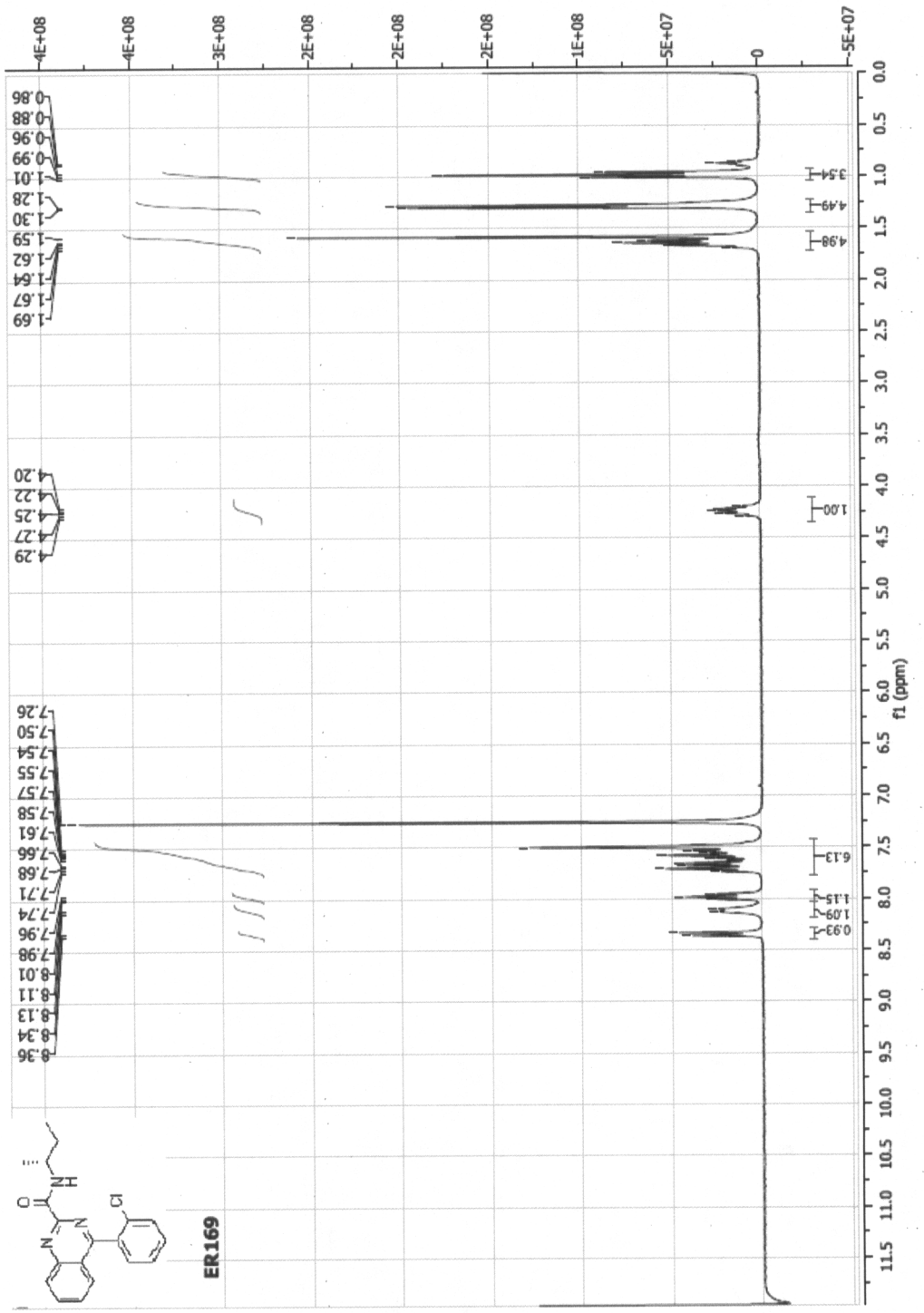
Appendix D: Representative Characterization Data for Precursor and Reference ER176

Precursor - ^{1}H NMR



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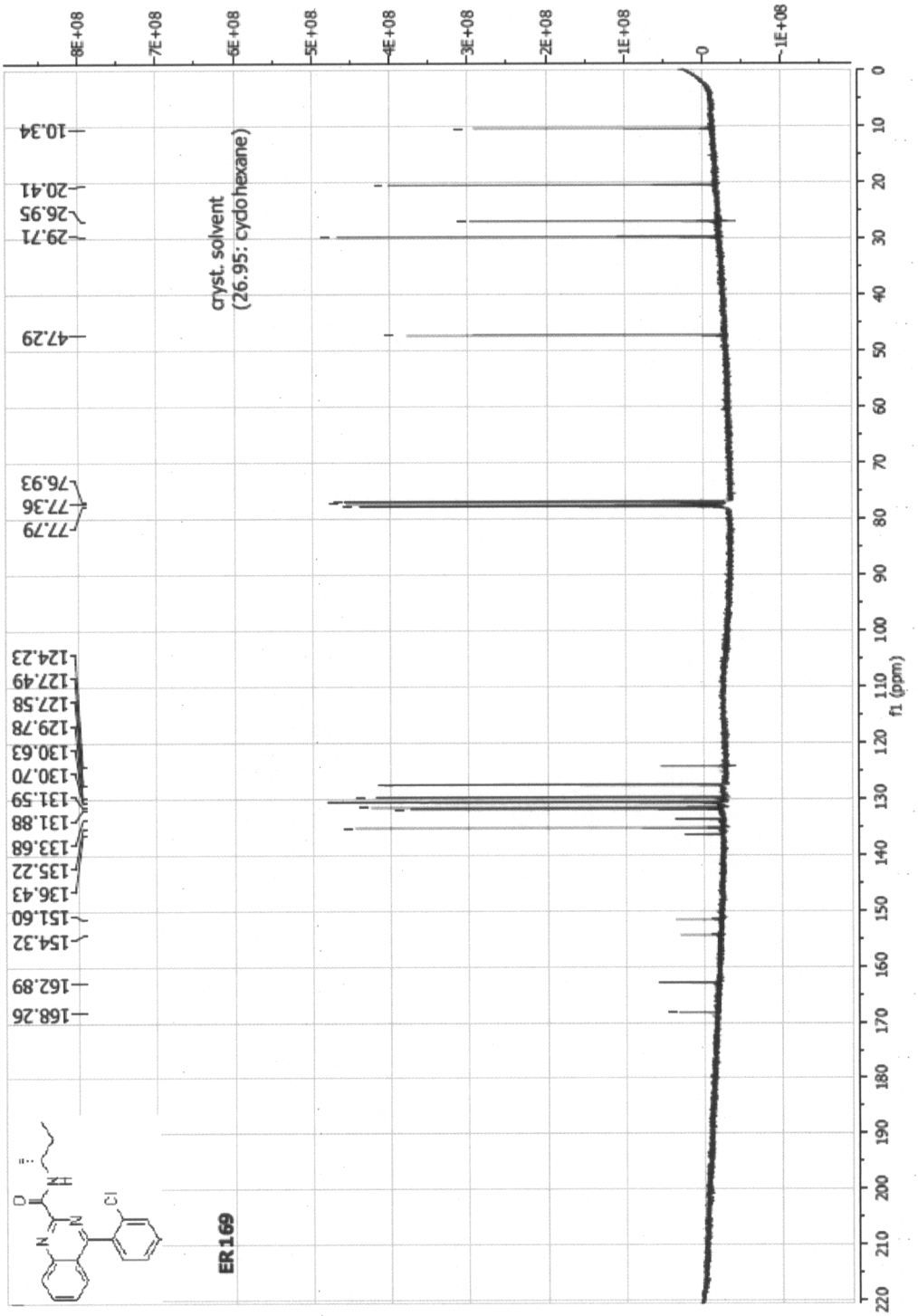
Precursor – ^{13}C NMR



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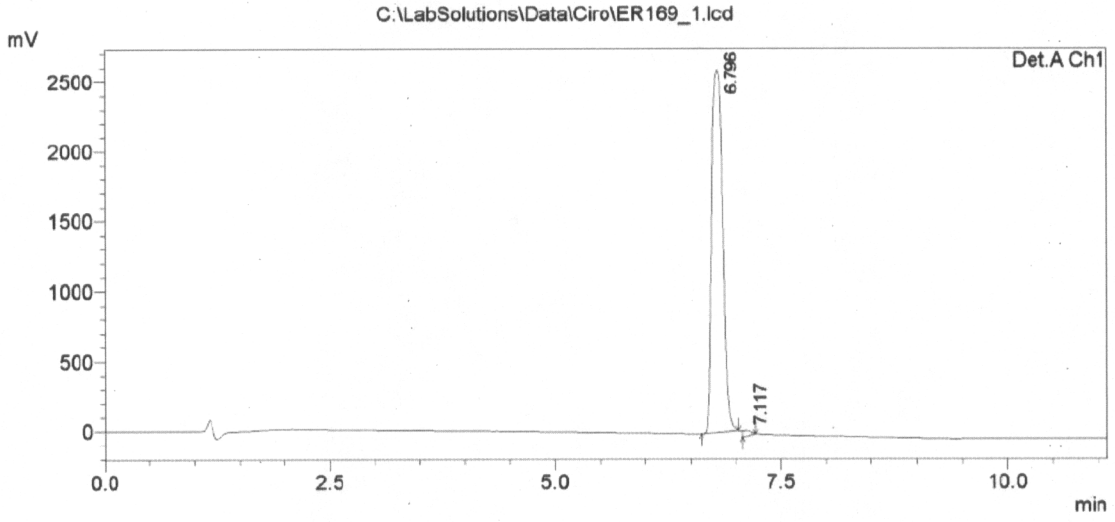
Precursor - HPLC

==== Shimadzu LCsolution Analysis Report ====

C:\LabSolutions\Data\Ciro\ER169_1.lcd

Acquired by	: Admin
Sample Name	: ER169_1
Sample ID	: ER169_1
Vail #	:
Injection Volume	: 20 uL
Data File Name	: ER169_1.lcd
Method File Name	: TSPO.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 03/09/2012 10.14.56
Data Processed	: 03/09/2012 11.11.36

<Chromatogram>



PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Height	Area %
1	6.796	21317468	2593125	98.901
2	7.117	236820	41495	1.099
Total		21554288	2634620	100.000

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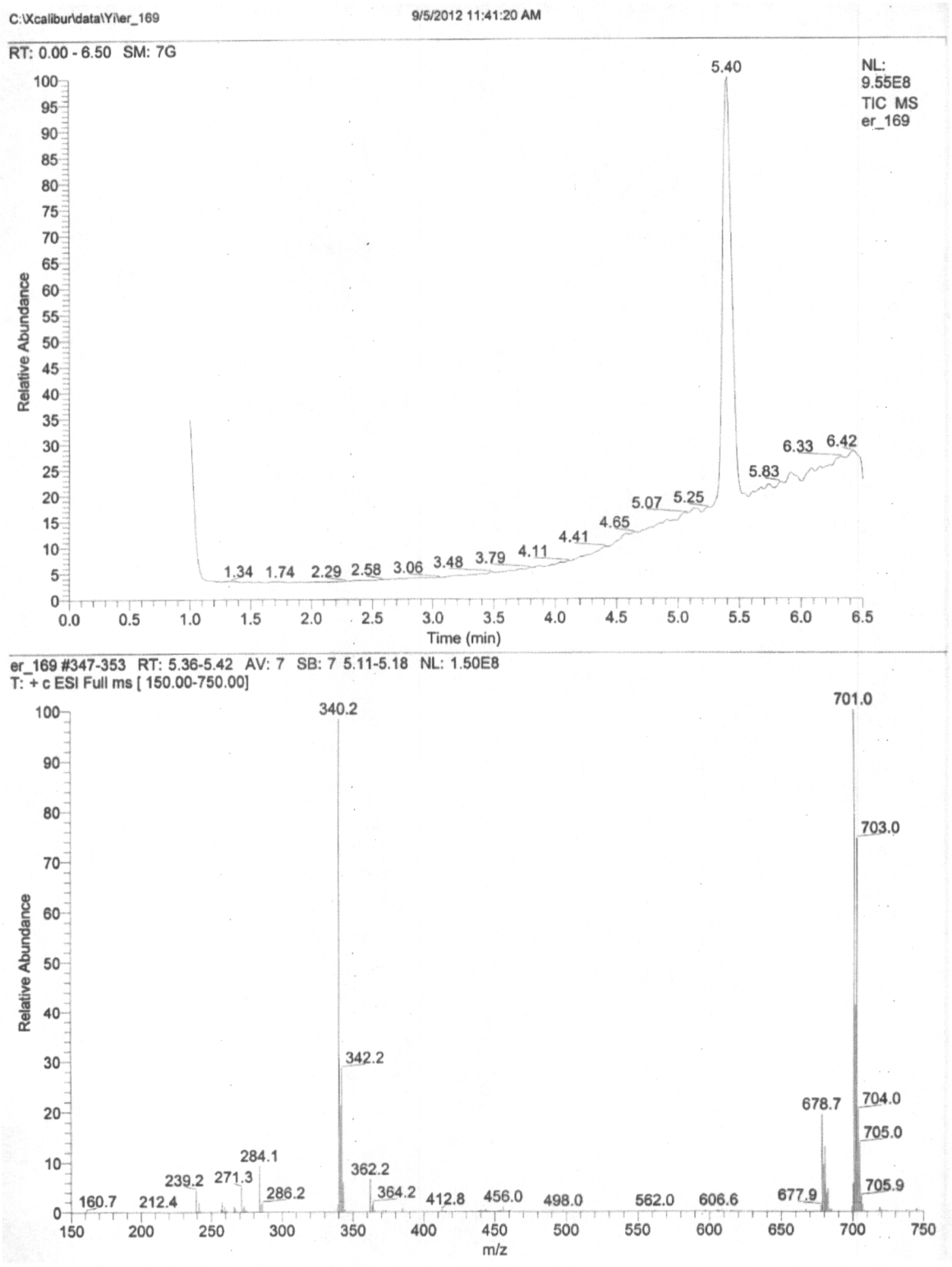
Precursor - LC/MS



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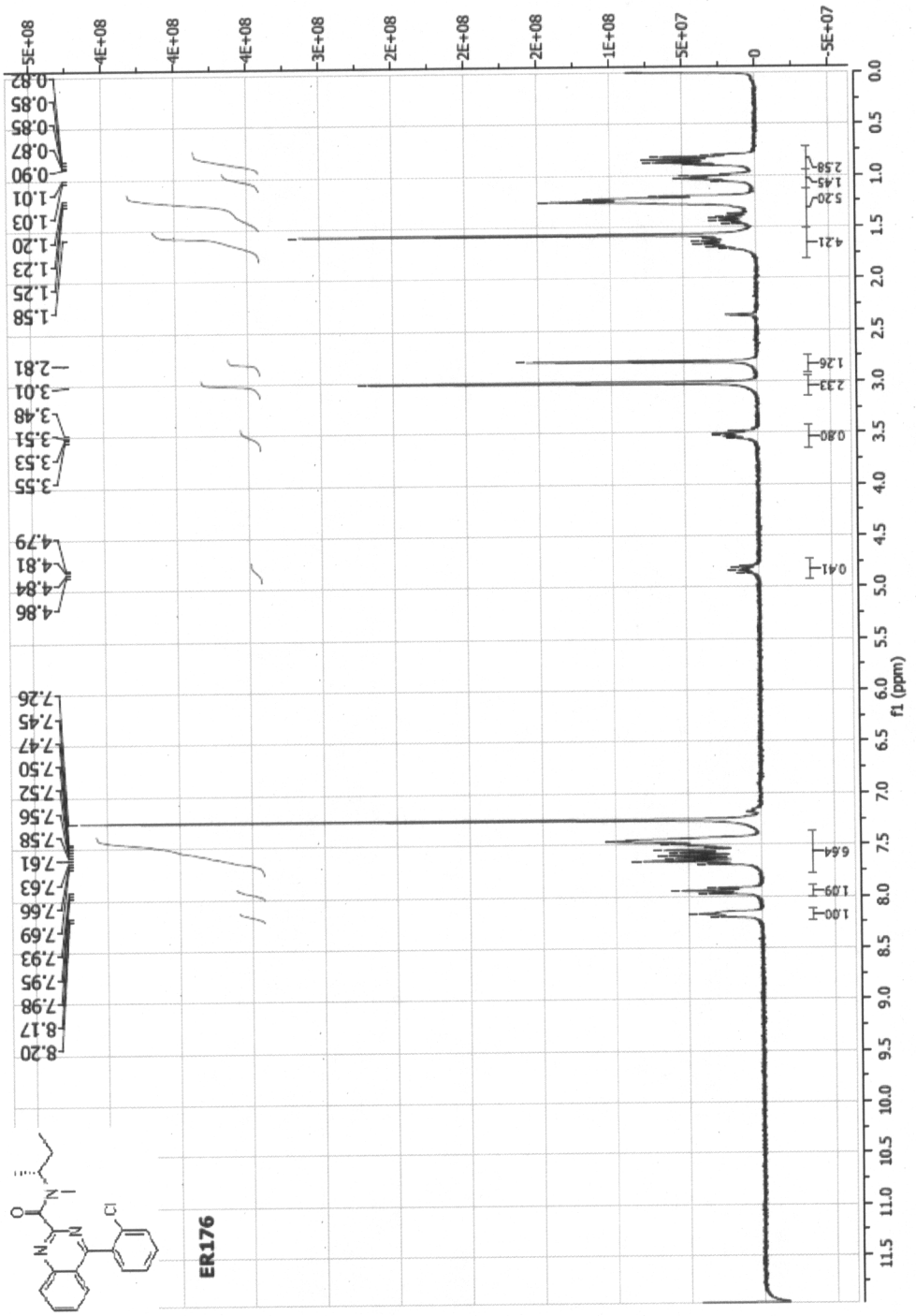
Reference Compound – ^{1}H NMR



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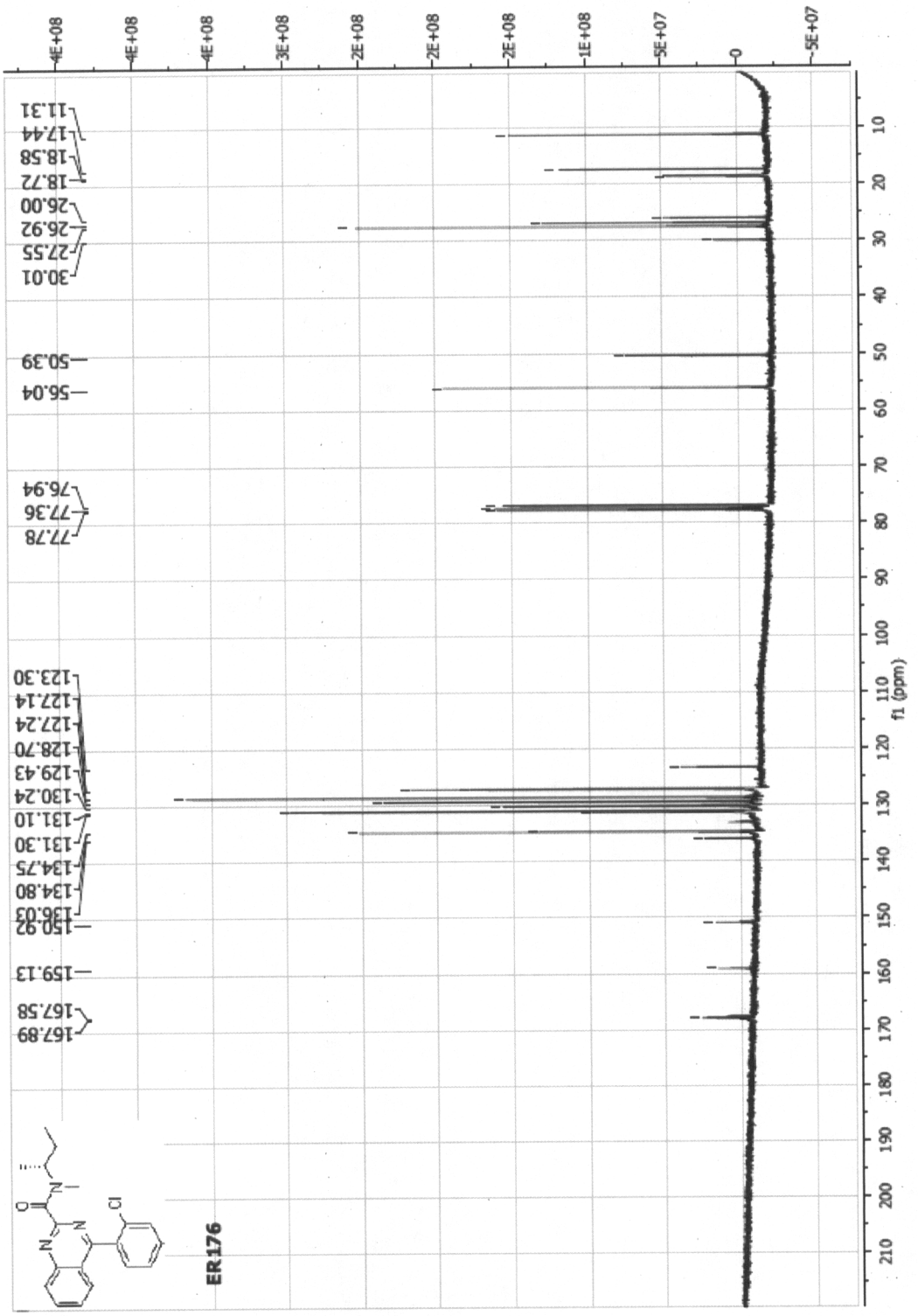
Reference Compound – ^{13}C NMR



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Reference Compound – HPLC

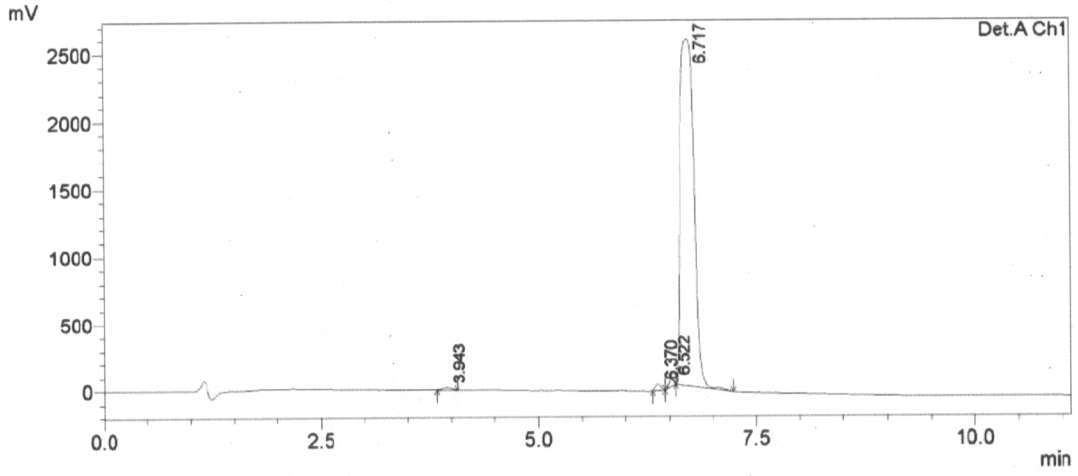
==== Shimadzu LCsolution Analysis Report ====

C:\LabSolutions\Data\Ciro\ER176_1.lcd

Acquired by	: Admin
Sample Name	: ER176_1
Sample ID	: ER176_1
Vail #	:
Injection Volume	: 20 uL
Data File Name	: ER176_1.lcd
Method File Name	: TSPO.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 03/09/2012 10.34.39
Data Processed	: 03/09/2012 10.45.49

<Chromatogram>

C:\LabSolutions\Data\Ciro\ER176_1.lcd



PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Height	Area %
1	3.943	158529	20125	0.556
2	6.370	203029	46769	0.713
3	6.522	197180	55632	0.692
4	6.717	27933512	2567058	98.039
Total		28492249	2689585	100.000

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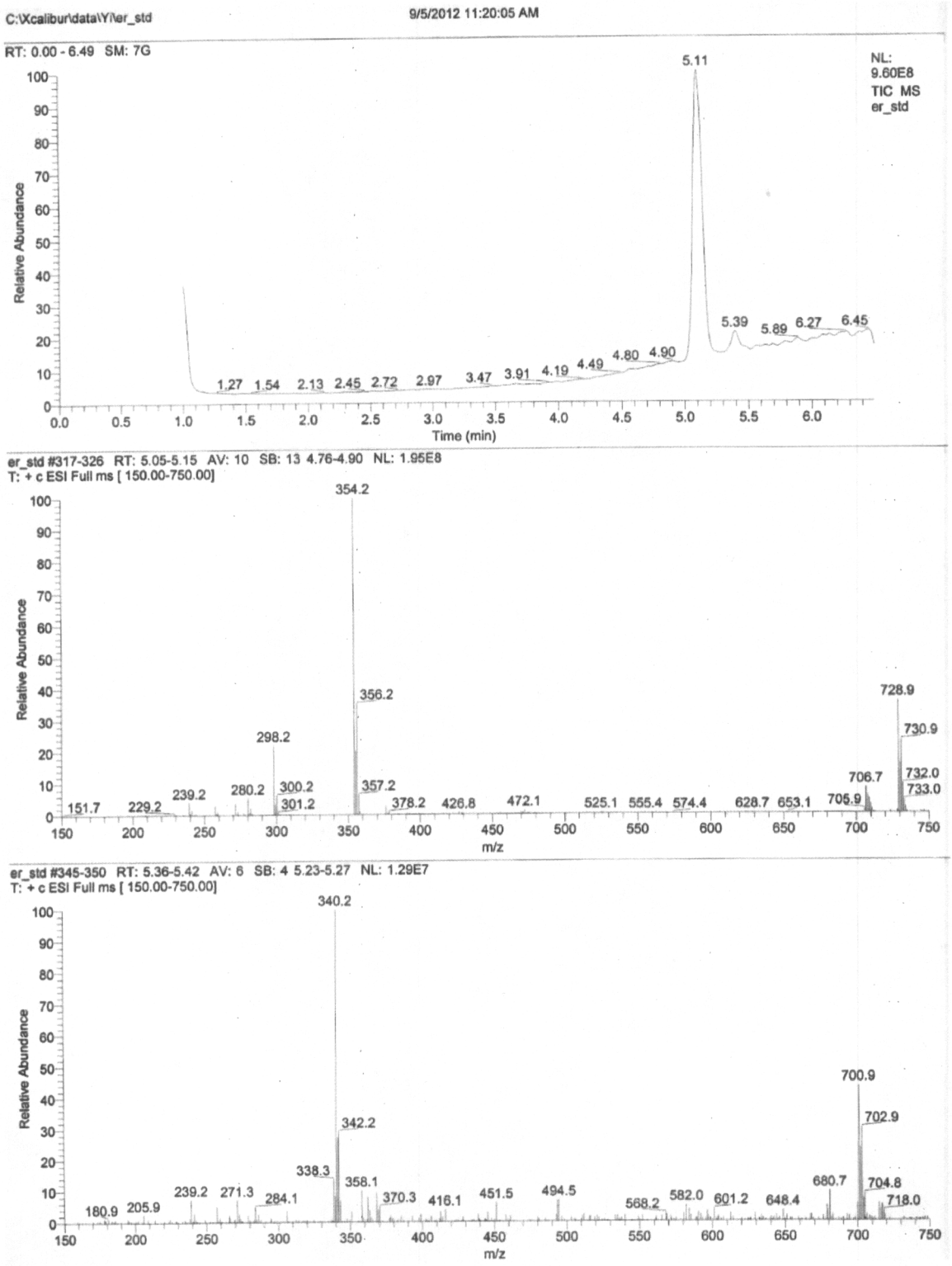
Reference Compound – LC/MS



PET Radiopharmaceutical Sciences Section,
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Appendix E: Synthia Cecilia Program Time Lists

index	Name	Sequence	Row_nr	Object	Function	Arg_1	Arg_2	Arg_3	Arg_4	Comments
58934	ER176 IND	#	1	Dout	Reset_All	#	#	#	#	#
58946	ER176 IND	#	2	#	Delay	5	#	#	#	Wait 2 s (for system to start)
58947	ER176 IND	#	3	Dilutor	Init	L	N	#	#	Initiating the dilutor
58935	ER176 IND	#	4	#	Delay	5	#	#	#	Wait 2 s (for system to start)
59054	ER176 IND	#	5	BCD	SET_Position	1	8	#	#	Set column switching valve to 8 on prep
58990	ER176 IND	#	100	#	Print	Initiating C-11 synthesis via MeI from GE	#	#	#	
58991	ER176 IND	#	300	Robot	Move_XYZ	A1	#	#	#	Position rinsing needle
58927	ER176 IND	#	400	Dilutor	Init	L	N	#	#	Initiating the dilutor
58949	ER176 IND	#	500	Robot	Switch_Valve	P	I	#	#	Activate MeI reactor 1
59022	ER176 IND	#	600	#	SetVariableName	ReactionTemperature	#	#	#	
58992	ER176 IND	#	600	Robot	Switch_Valve	A	I	#	#	Activate MeI reactor 1
59023	ER176 IND	#	610	#	UserInput	Set the reaction temperature.	ReactionTemperature	#	#	
58993	ER176 IND	#	620	Oven	Set_Temperature	6	ReactionTemperature	#	#	
[illegible]024	ER176 IND	#	630	#	SetVariableName	Reactiontime	#	#	#	
58950	ER176 IND	#	640	#	UserInput	Set reaction time in seconds.	Reactiontime	#	#	
59025	ER176 IND	#	650	#	Print	-	#	#	#	
58994	ER176 IND	#	660	#	Delay	3	#	#	#	
58995	ER176 IND	#	800	Oven	Set_Temperature	8	80	#	#	Set evaporator temperature
58951	ER176 IND	#	900	Dout	Off	34	#	#	#	Sterile needles go up
58952	ER176 IND	#	910	Robot	Switch_Valve	A	L	#	#	Analytical injection on load
58929	ER176 IND	#	920	#	Attention	Place 70/30 EtOH/water in vial F31. Clean analytical Injection port. And Hit Okay.	#	#	#	
58996	ER176 IND	#	930	Robot	Switch_Valve	A	I	#	#	prep injection valve set on Inject
58931	ER176 IND	#	950	Robot	Move_XYZ0	F31	#	#	#	
59026	ER176 IND	#	955	Dilutor	Aspirate	L	300	50	N	Rinsing the needle
58997	ER176 IND	#	957	Robot	Move_XYZ	F31	#	#	#	
59027	ER176 IND	#	959	Dilutor	Aspirate	L	3000	50	N	pulling up 3 ml of 70/30 EtOH/water
59028	ER176 IND	#	961	Robot	Move_XYZ	B11	#	#	#	
58998	ER176 IND	#	963	Robot	Switch_Valve	P	L	#	#	Activate MeI reactor 1
59029	ER176 IND	#	965	Dilutor	Dispense	L	3000	20	N	pulling up 3 ml of 70/30 EtOH/water
58999	ER176 IND	#	967	Dilutor	Aspirate	L	5000	20	R	pulling up 3 ml of 70/30 EtOH/water
[illegible]932	ER176 IND	#	969	Dilutor	Dispense	L	5000	20	N	pulling up 3 ml of 70/30 EtOH/water
58953	ER176 IND	#	971	Robot	Switch_Valve	P	I	#	#	Activate MeI reactor 1
58954	ER176 IND	#	1000	#	Delay	1	#	#	#	Wait 2 s

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index	Name	Sequence	Row_nr	Object	Function	Arg_1	Arg_2	Arg_3	Arg_4	Comments
59030	ER176 IND	#	1200	Dout	Off	36	#	#	#	Closing the trapdoor
59031	ER176 IND	#	1350	Dout	On	35	#	#	#	Sterile vial slider out from sterile station
59000	ER176 IND	#	1400	Dout	On	4	#	#	#	Condition trap tubings
58858	ER176 IND	#	1500	Flow	Set_Flow	1	20	200	#	Setting nitrogen flow to 20 ml/min
59032	ER176 IND	#	1600	Dout	Rotor_Valve	6	#	#	#	Bypass MeI reactors
58955	ER176 IND	#	1799	#	Attention	Check that all reagents are in position! Remove slider so it doesn't overheat	#	#	#	Wait 2 s
59001	ER176 IND	#	1900	#	Print	Priming the dilutor.	#	#	#	
58871	ER176 IND	#	2000	Dilutor	Init	L	N	#	#	Initiating the dilutor
58838	ER176 IND	#	2600	Dilutor	Aspirate	L	2000	50	R	Priming the dilutor / Rinse needle
59033	ER176 IND	#	2700	Dilutor	Dispense	L	2000	20	N	Priming the dilutor / Rinse needle
59002	ER176 IND	#	2800	Robot	Move_XYZ	A2	#	#	#	Position rinsing the top of the needle
[illegible]56	ER176 IND	#	2900	Dilutor	Aspirate	L	1000	50	R	Priming the dilutor / Rinse needle
59003	ER176 IND	#	3000	Dilutor	Dispense	L	1000	10	N	Priming the dilutor / Rinse needle
58879	ER176 IND	#	3500	#	Print	-	#	#	#	
59004	ER176 IND	#	3600	Robot	Home	#	#	#	#	
58957	ER176 IND	#	3610	#	Ask	Click YES button to clean rotavap or NO button to skip cleaning step for rotary evaporation?	No_rotavap_wash	#	#	
58889	ER176 IND	#	3650	#	Attention	Connect the rinse tubing at the sterile station.	#	#	#	
58890	ER176 IND	#	3660	Dout	On	44	#	#	#	
58891	ER176 IND	#	3665	Dout	On	45	#	#	#	
58875	ER176 IND	#	3700	#	Print	Aspirating the needle free from eluent.	#	#	#	
58842	ER176 IND	#	3800	Dilutor	Aspirate	L	1200	5	N	Aspirating the needle free from eluent
58843	ER176 IND	#	3900	#	Attention	Load evaporator washing solutions!	#	#	#	
58877	ER176 IND	#	4000	#	Print	Washing the rotary evaporator with 70% ethanol.	#	#	#	
58881	ER176 IND	#	4100	Dout	Off	45	#	#	#	
[illegible]82	ER176 IND	#	4200	Dout	Off	44	#	#	#	
[illegible]862	ER176 IND	#	4300	Dout	On	41	#	#	#	
58861	ER176 IND	#	4400	Dout	On	46	#	#	#	
58893	ER176 IND	#	4550	Vacuum	Start	5	#	#	#	Start vacuum pump

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index	Name	Sequence	Row_nr	Object	Function	Arg_1	Arg_2	Arg_3	Arg_4	Comments
58901	ER176 IND	#	4555	Robot	Move_XYZ	F12	#	#	#	Position washing solution
58900	ER176 IND	#	4560	#	Delay	10	#	#	#	
58895	ER176 IND	#	4600	Dout	On	37	#	#	#	Elevate evaporator heating bath
58863	ER176 IND	#	4700	Dout	On	57	#	#	#	Evap rotation on
58864	ER176 IND	#	4800	#	Delay	5	#	#	#	
58896	ER176 IND	#	4820	#	Attention	Stop Washing.	#	#	#	
58897	ER176 IND	#	4830	Dout	Off	57	#	#	#	Evap rotation off
58898	ER176 IND	#	4840	#	Delay	1	#	#	#	Wait 40 s
58899	ER176 IND	#	4845	Dout	Off	37	#	#	#	Lower evaporator heating bath
58894	ER176 IND	#	4850	Vacuum	Stop	#	#	#	#	Stop vacuum pump
58902	ER176 IND	#	4855	#	Delay	4	#	#	#	
58865	ER176 IND	#	4900	Dout	Off	46	#	#	#	
58872	ER176 IND	#	5100	Dout	Off	41	#	#	#	
58866	ER176 IND	#	5150	Dout	On	45	#	#	#	
58903	ER176 IND	#	5160	#	Delay	2	#	#	#	
58873	ER176 IND	#	5200	Dout	On	44	#	#	#	
58870	ER176 IND	#	5400	Robot	Home	#	#	#	#	
58883	ER176 IND	#	5500	#	Delay	10	#	#	#	
[illegible]78	ER176 IND	#	5600	#	Print	-	#	#	#	
58844	ER176 IND	#	5700	#	Ask	Do you want to wash the rotary evaporator with ethanol once more? If Yes, load more EtOH washing solution.	Water_Wash	#	#	
58888	ER176 IND	#	5800	#	Jump	Ethanol_Wash	#	#	#	
58885	ER176 IND	#	5900	#	Print	Washing the evaporator with sterile water.	#	#	#	
58904	ER176 IND	#	6000	Dout	Off	45	#	#	#	
58905	ER176 IND	#	6010	Dout	Off	44	#	#	#	
58906	ER176 IND	#	6020	Dout	On	41	#	#	#	
58907	ER176 IND	#	6030	Dout	On	46	#	#	#	
58908	ER176 IND	#	6040	Vacuum	Start	5	#	#	#	Start vacuum pump
58909	ER176 IND	#	6050	Robot	Move_XYZ	F11	#	#	#	Position washing solution
58910	ER176 IND	#	6060	#	Delay	10	#	#	#	
58911	ER176 IND	#	6070	Dout	On	37	#	#	#	Elevate evaporator heating bath
58912	ER176 IND	#	6080	Dout	On	57	#	#	#	Evap rotation on
58913	ER176 IND	#	6090	#	Delay	5	#	#	#	
58914	ER176 IND	#	6100	#	Attention	Stop Washing.	#	#	#	
58915	ER176 IND	#	6110	Dout	Off	57	#	#	#	Evap rotation off
58916	ER176 IND	#	6120	#	Delay	1	#	#	#	Wait 40 s
58917	ER176 IND	#	6130	Dout	Off	37	#	#	#	Lower evaporator heating bath
[illegible]18	ER176 IND	#	6140	Vacuum	Stop	#	#	#	#	Stop vacuum pump
[illegible]919	ER176 IND	#	6150	#	Delay	4	#	#	#	
58920	ER176 IND	#	6160	Dout	Off	46	#	#	#	
58921	ER176 IND	#	6170	Dout	Off	41	#	#	#	

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index	Name	Sequence	Row_nr	Object	Function	Arg_1	Arg_2	Arg_3	Arg_4	Comments
58922	ER176 IND	#	6180	Dout	On	45	#	#	#	
58923	ER176 IND	#	6190	#	Delay	2	#	#	#	
58924	ER176 IND	#	6200	Dout	On	44	#	#	#	
58925	ER176 IND	#	6210	Robot	Home	#	#	#	#	
58926	ER176 IND	#	6220	#	Delay	10	#	#	#	
58886	ER176 IND	#	7500	#	Print	-	#	#	#	
58887	ER176 IND	#	7600	#	Ask	Is the evaporator clean? If No, load new Water and EtOH washing solutions.	Ethanol_Wash	#	#	
58857	ER176 IND	#	7650	Oven	Set_Temperature	8	80	#	#	Set evaporator temperature
59034	ER176 IND	#	7700	#	Print	Positioning the SPE slider.	#	#	#	
59035	ER176 IND	#	7800	Robot	Move_XYZ	I1	#	#	#	Positioning the SPE slider
58958	ER176 IND	#	7900	Robot	Move_XY	I6	800	#	#	Positioning the SPE slider
59036	ER176 IND	#	8000	Robot	Move_XYZ	I7	#	#	#	Positioning the SPE slider
58839	ER176 IND	#	8100	Robot	Move_XY	I8	800	#	#	Positioning the SPE slider
58959	ER176 IND	#	8200	#	Print	Rinsing the needle.	#	#	#	
[illegible]037	ER176 IND	#	8300	Robot	Move_XYZ	A1	#	#	#	Position rinsing needle
[illegible]960	ER176 IND	#	8400	Dilutor	Aspirate	L	700	50	R	Rinsing the needle
58884	ER176 IND	#	8500	Dilutor	Dispense	L	1900	20	N	Rinsing the needle
59005	ER176 IND	#	8600	Robot	Home	#	#	#	#	Position home
59038	ER176 IND	#	8700	Robot	Move_XYZ0	F10	#	#	#	Positioning the SPE slider
58841	ER176 IND	#	8800	Dilutor	Aspirate	L	100	3	N	Aspirating air plug
58840	ER176 IND	#	8900	#	Print	Dispensing 5 mL sterile buffer solution.	#	#	#	
58845	ER176 IND	#	9400	#	Print	Rinsing the needle.	#	#	#	
58846	ER176 IND	#	9500	Robot	Move_XYZ	A1	#	#	#	Position rinsing needle
58847	ER176 IND	#	9600	Dilutor	Aspirate	L	700	50	R	Rinsing the needle
58848	ER176 IND	#	9700	Dilutor	Dispense	L	800	20	N	Rinsing the needle
58849	ER176 IND	#	9800	Robot	Home	#	#	#	#	Position home
58867	ER176 IND	#	9900	Dout	Off	45	#	#	#	Stop conditioning the sterile and evqaporator tubings
58868	ER176 IND	#	10000	#	Delay	5	#	#	#	Stop conditioning the sterile and evqaporator tubings
58869	ER176 IND	#	10100	Dout	Off	44	#	#	#	Stop conditioning the sterile and evqaporator tubings
58876	ER176 IND	#	12600	#	Print	Rinsing the needle.	#	#	#	
58961	ER176 IND	#	12700	Robot	Move_XYZ	A1	#	#	#	Position rinsing needle
58850	ER176 IND	#	12800	Dilutor	Aspirate	L	1000	50	R	Rinsing the needle
58962	ER176 IND	#	12900	Dilutor	Dispense	L	1000	20	N	Rinsing the needle
58963	ER176 IND	#	13000	Robot	Move_XYZ	A2	#	#	#	Position rinsing the top of the needle
[illegible]964	ER176 IND	#	13100	Dilutor	Aspirate	L	500	50	R	Rinsing the needle
58965	ER176 IND	#	13200	Dilutor	Dispense	L	500	10	N	Rinsing the needle
59039	ER176 IND	#	13300	Dout	Off	33	#	#	#	Trap needles go up

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index	Name	Sequence	Row_nr	Object	Function	Arg_1	Arg_2	Arg_3	Arg_4	Comments
58966	ER176 IND	#	13400	#	Print	Positioning the reaction slider.	#	#	#	
58851	ER176 IND	#	13500	Robot	Move_XYZ	I1	#	#	#	Positioning the reaction slider
58967	ER176 IND	#	13550	Dout	Off	33	#	#	#	Trap needles go up (if trap station was not ready for use
58968	ER176 IND	#	13600	Robot	Move_XY	I2	800	#	#	Positioning the reaction slider
58969	ER176 IND	#	13800	Robot	Move_XYZ	I3	#	#	#	Positioning the reaction slider
59040	ER176 IND	#	13900	Robot	Move_XY	I4	800	#	#	Positioning the reaction slider
59006	ER176 IND	#	14000	Robot	Move_XYZ0	F10	#	#	#	Position over washing solution reservoir
58970	ER176 IND	#	14100	#	Print	-	#	#	#	
58880	ER176 IND	#	14300	#	Attention	Make sure Ge MeI output is hooked into position 2 of synthia. Place the precursor v-vial in slider position 2, and press Ok!	#	#	#	
59041	ER176 IND	#	14400	#	Attention	Set Step 8 of GE MeI program to 3 min prior to EOB. Load slider/precursor just before MeI release. Press OK when this is done	#	#	#	
59042	ER176 IND	#	14700	Robot	Move_XYZ	I1	#	#	#	Positioning the reaction slider
59007	ER176 IND	#	14800	Robot	Move_XY	I2	800	#	#	Positioning the reaction slider
59043	ER176 IND	#	14900	#	Delay	1	#	#	#	Wait 1 s
59008	ER176 IND	#	14950	Dout	Off	4	#	#	#	N2 gas flow not to trap station
59044	ER176 IND	#	15000	Dout	On	33	#	#	#	Trap needles go down
58971	ER176 IND	#	15100	Robot	Move_XYZ0	F10	#	#	#	Removing aspec arm
58972	ER176 IND	#	15200	#	Ask	Is the trap station ready for use?	No_trap_ready	#	#	
59045	ER176 IND	#	16600	#	Print	Ready for synthesis/EOB	#	#	#	
58859	ER176 IND	#	35300	Robot	Home	#	#	#	#	
58973	ER176 IND	#	35350	#	Attention	Press OK when C-11 MeI begins to release	#	#	#	
59046	ER176 IND	#	35400	Dout	On	70	#	#	#	Trap needles go down
58974	ER176 IND	#	35600	#	Attention	Press OK when C-11 MeI maximizes in precursor solution	#	#	#	
59009	ER176 IND	#	35650	Dout	On	26	#	#	#	Trap needles go down
58930	ER176 IND	#	35660	Dout	Off	70	#	#	#	Trap needles go up
58975	ER176 IND	#	36400	#	Print	Moving reaction vial to the oven.	#	#	#	
[illegible]047	ER176 IND	#	36500	Dout	Off	33	#	#	#	Trap needles go up
58852	ER176 IND	#	36600	Robot	Move_XYZ	I1	#	#	#	Positioning the reaction slider
58976	ER176 IND	#	36700	Robot	Move_XY	I2	800	#	#	Positioning the reaction slider

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index	Name	Sequence	Row_nr	Object	Function	Arg_1	Arg_2	Arg_3	Arg_4	Comments
58977	ER176 IND	#	36800	Robot	Move_XYZ	I3	#	#	#	Positioning the reaction slider
58853	ER176 IND	#	36900	Robot	Move_XY	I4	800	#	#	Transferring the reaction vial to the oven
58928	ER176 IND	#	36910	Robot	Move_XY	I5	800	#	#	
59010	ER176 IND	#	36920	Robot	Move_R	30	20	Y	-	
58978	ER176 IND	#	36930	Robot	Move_R	60	20	Y	+	
59011	ER176 IND	#	36940	Robot	Move_R	30	20	Y	-	
58939	ER176 IND	#	36954	Robot	Set_Speed	1250	700	#	#	#
58979	ER176 IND	#	37300	Oven	Set_Temperature	1	18	#	#	Turn off 1st MeI reactor oven
59012	ER176 IND	#	37400	Oven	Set_Temperature	2	18	#	#	Turn off 2nd MeI reactor oven
59013	ER176 IND	#	37500	Oven	Set_Temperature	3	18	#	#	Turn off 3rd MeI reactor oven
58860	ER176 IND	#	37600	Oven	Set_Temperature	4	18	#	#	Turn off 4th MeI reactor oven
59048	ER176 IND	#	37700	Oven	Set_Temperature	5	18	#	#	Turn off 5th MeI reactor oven
59049	ER176 IND	#	37800	Robot	Home	#	#	#	#	Position home
58980	ER176 IND	#	37900	#	Print	Heating the reaction mixture	#	#	#	
58981	ER176 IND	#	38000	#	Delay	Reactiontime	#	#	#	Alkylation reaction time (s)
59014	ER176 IND	#	38100	Oven	Set_Temperature	6	18	#	#	Turn off reaction oven
59015	ER176 IND	#	38200	#	Print	Diluting the reaction mixture with 500uL water.	#	#	#	
58982	ER176 IND	#	38300	Dilutor	Aspirate	L	500	20	R	Asp. Dilution volume
58983	ER176 IND	#	38400	Robot	Move_XYZ	E12	#	#	#	Position reaction vial in oven
58854	ER176 IND	#	38500	Dilutor	Dispense	L	500	5	N	Disp. dilution vol
59050	ER176 IND	#	38600	#	Print	Injecting on the preparative HPLC system.	#	#	#	
59051	ER176 IND	#	38700	Robot	Move_XYZ0	E12	#	#	#	Position over reaction oven
58855	ER176 IND	#	38800	Dilutor	Aspirate	L	150	3	N	Aspirating air plug
58984	ER176 IND	#	38900	Robot	Move_XYZ	E12	#	#	#	Position reaction vial in oven
58856	ER176 IND	#	39000	Dilutor	Aspirate	L	950	10	N	Asp. reaction mixture + dil. Volumev
58985	ER176 IND	#	39100	Robot	Move_XYZ	B11	#	#	#	Position preparative injection port
59016	ER176 IND	#	39300	Robot	Switch_Valve	P	L	#	#	Preparative injection valve set on Load
59017	ER176 IND	#	39400	Dilutor	Dispense	L	950	5	N	Load reaction mixture + dil. Volume on prep injection valve
59052	ER176 IND	#	39410	Dilutor	Dispense	L	150	5	N	Dispensing reaction mixture in preparative injection port
59018	ER176 IND	#	39411	Dilutor	Aspirate	L	500	5	R	Aspirating water frpm reservoir...
58933	ER176 IND	#	39412	Dilutor	Dispense	L	500	5	N	Dispensing reaction mixture in preparative injection port
58986	ER176 IND	#	39420	#	Delay	1	#	#	#	
59019	ER176 IND	#	39500	Robot	Switch_Valve	P	I	#	#	Preparative injection valve set on Inject
020	ER176 IND	#	39600	#	Print	Starting the preparative HPLC system.	#	#	#	
58987	ER176 IND	#	39700	Dout	On	56	#	#	#	Signalling to start prep HPLC

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index	Name	Sequence	Row_ nr	Object	Function	Arg_1	Arg_2	Arg_3	Arg_4	Comments
										system
58874	ER176 IND	#	39800	#	Delay	2	#	#	#	Wait 2 s (for system to start)
59053	ER176 IND	#	39900	Dout	Off	56	#	#	#	Stop signalling to prep HPLC
58942	ER176 IND	Buffer_to_ evaporation	41295	#	#	#	#	#	#	
58945	ER176 IND	Buffer_to_ evaporation	41295	#	#	#	#	#	#	
58941	ER176 IND	Evaporation	41285	#	#	#	#	#	#	
58944	ER176 IND	Evaporation	41285	#	#	#	#	#	#	
58940	ER176 IND	Prep_Aspirate	41275	#	#	#	#	#	#	
58943	ER176 IND	Prep_Aspirate	41275	#	#	#	#	#	#	
58988	ER176 IND	Prep_Fraction	41265	#	#	#	#	#	#	
59021	ER176 IND	Prep_Fraction	41265	#	#	#	#	#		

[^{11}C]ER176 Injection: Master Batch Record

PET Radiopharmaceutical Sciences Section,
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Date of review: 8/28/2012

Approved by: V.W. Pike Initials: VWP Date: 08/28/12

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

Batch # ER176-_____ Date: _____

Reagents/solvents/supplies	Lot/Exp	Purpose	Amount used	Date of Preparation/ Date Opened
Dimethyl sulfoxide, anhydrous		Solvent	0.4 mL	
Potassium hydroxide, 85%		Reagent Base	mg	
N-desmethyl-ER176		Precursor	mg	
Ethyl alcohol, USP 200 proof		Cleaning	As required	
Sterile Saline for Injection, USP	/	Formulation	10.0 mL	Single Use
Ethanol, dehydrated	/	Formulation	0.5 ml	Single Use
Sterile vial 10 mL		Dose vial	1	Single Use
Sterile Millex-GV filter (vent filter, 0.22 µm, 4 mm diameter)	/	Formulation	1	Single Use
Sterile Millex-MP filter (sterilization filter, 0.22 µm, 25 mm diameter)	/	Formulation	1	Single Use
Sterile needle (21 gauge; 2") for sterile filtration, 2 each and [^{11}C]MeI transfer, 2 each		Formulation	4	Single Use
Sterile needle (20 gauge; 1.5 inches long) for sterile vent		Formulation	1	Single Use
Water, HPLC grade	/	HPLC mobile phase	As required	
Acetonitrile, HPLC grade		HPLC mobile phase	As required	
Methanol, HPLC grade		HPLC mobile phase	As required	
Ammonium hydroxide, 1N		Mobile phase modifier	1 mL / 1 L water	
HPLC column (analytical, Phenomenex, C-18 Luna; 10 µm; 4.6 mm × 250 mm)		Analytical HPLC	1	
HPLC column (semi-prep, Waters, RP-18 XTerra; 10 µm; 7.8 mm × 300 mm)		Prep HPLC	1	
1 mM ammonium hydroxide (aq)	/	Prep HPLC mobile phase	1L nominal	
MeOH:water, 74:26	/	Analytical HPLC mobile phase	1L nominal	

[^{11}C]ER176 Injection: Master Batch Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Key operation	Initials	Comment/SOP #
Check all gas valves are open and that pressure on regulators are 60, 12 and 22 p.s.i. for nitrogen, helium and hydrogen, respectively		
6-way valve in NEMA box is set to hot-cell 3 and 3-way valve is on "cryo" position		
Check gas collection valve is on "fill" position		
Run prep sequence on GE Microlab Mel box		
Check flow in RMA, RMB, RMC		
Check the helium flow rate. Typical flow is 15 to 25 mL/min		
Check integrity of GE Mel box by running leak check 1		
Install clean transfer and vent needles at the Trapping station.		
Verify the ionization chamber with ^{57}Co and ^{137}Cs standards		
Verify balance using a 10-g NIST traceable weight (acceptable range 9.9−10.1 g)		Weight: _____g
Record weight of sterile empty vial on QC Form.		
Remove slider and empty reaction oven		
Open Synthia AutoRad Software		
Load Recipe **ER176IND**		
Enter Reaction Temperature (80 °C) and Time (300 s) when prompted		
Ensure preparative column switching valve is set to ER176 column and switch is set to "Prep" and install preparative mobile phase		
Equilibrate the preparative column with 37/63 acetonitrile/1 mM ammonium hydroxide at 8.0 mL/min. Check for leaks. Pressure should be about 3700 p.s.i.		
Ensure analytical column switching valve is set to ER176 column and switch is set to "Analytical" and install analytical mobile phase		
Equilibrate the analytical column with 74/26 MeOH/H_2O at 2.5 mL/min. Check for leaks. Pressure should be about 2400 p.s.i.		
Inject ER176 standard and clean analytical injection port.		
Test 32Karat Beckman HPLC data acquisition interface box, UV, and PIN diode detector by initiating data collection		
Connect and wash the product collection line by open the collection valve and flush the Prep mobile phase through the line for at least three minutes.		
Clean all transfer tubing (20-mL column, HPLC fraction collection line, saline inlet line) with USP ethanol and flush dry.		
Verify vacuum integrity. Turn on pump; gauge should read approximately 28 inches of mercury (67mBar)		
Connect a 10-mL syringe containing 5% ethanol in saline to end of addition line.		
Fill heating bath with water and set to 80 °C		

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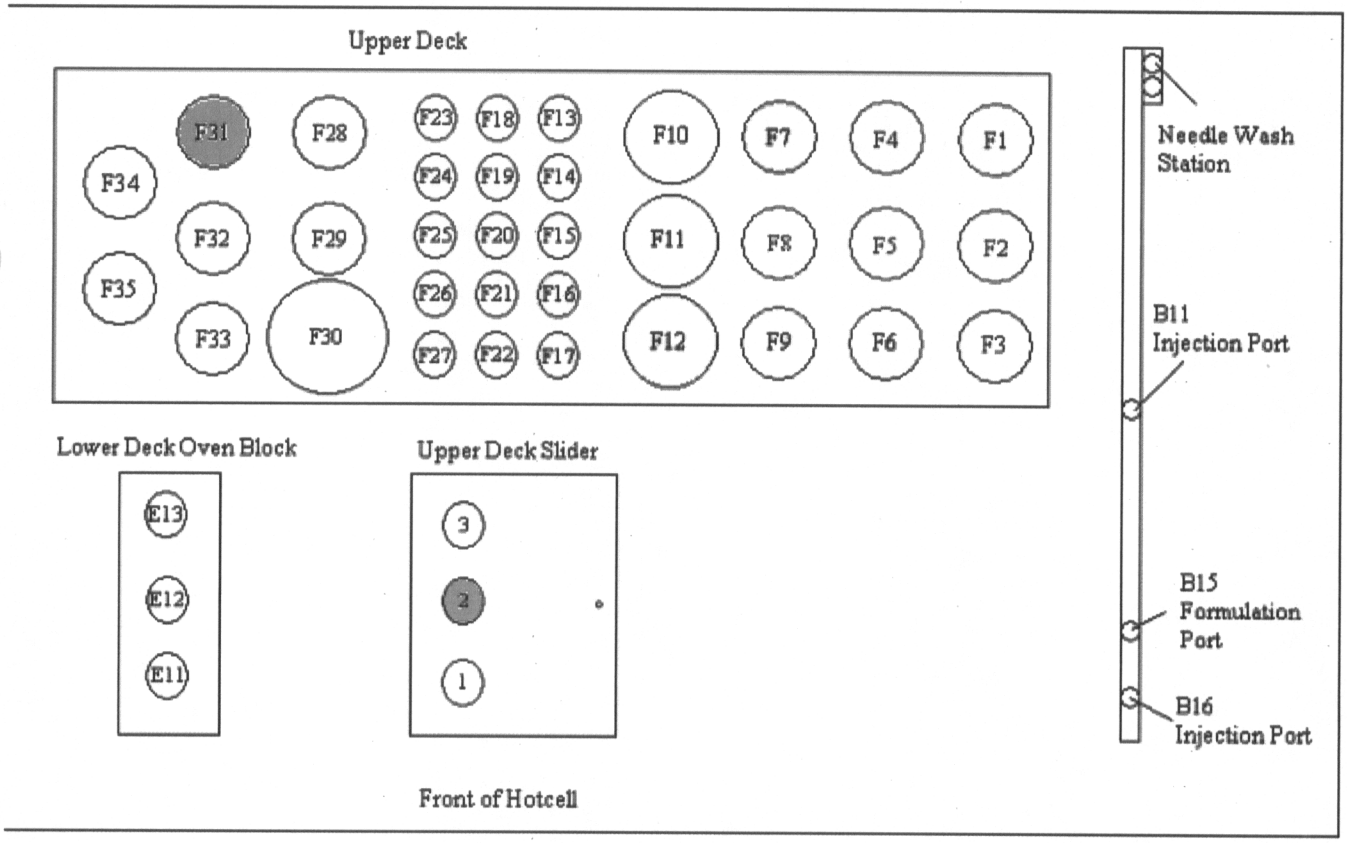
Date of review: 8/28/2012

Check that the dry ice traps are full		
Record weight of sterile empty vial; prepare and install sterile dose vial unit.		
Check that all waste reservoirs are sufficiently empty		
Prepare a vial for residual solvent analysis and place in the formulation hot cell		
Prepare the endotoxin unit for operation		
Prepare solution of ER176 precursor (0.6 ± 0.06 mg) / KOH (1.2 ± 0.2 mg) in DMSO (0.4 mL), crimp seal and place in Slider position 2 underneath vent needle when prompted.		

Figure 1: Diagram of Synthia Deck Layout



Position	Tube Type	Solvent / Material
F31	10 mL round bottom	70:30 Absolute Ethanol: HPLC grade Water
Slider Position 2	Reaction Vial	Precursor, KOH, DMSO

PET Radiopharmaceutical Sciences Section,
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Date of review: 8/28/2012

Summary:

Cyclotron, run #	
End of bombardment	
Beam current	μA
Bombardment time	min
Final formulated product in dose calibrator	mCi at

Chemist:	Signature:	Date

[^{11}C]ER176 Injection: Quality Control Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 2/18/2014

Approved by: V. W. Pike Initials: V.WP Date: 02/18/14
Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Batch # ER176-_____ Date: _____

Quality Control Instrument and Materials Verification			
Verification of Ionization Chamber	**Test Data**	**Acceptance Criteria**	**Acceptance Criteria Met**
^{137}Cs source reference ID: 970-31-4 204 μCi on 3/1/2003 Expected radioactivity _____μCi Acceptable Range: _____μCi to _____μCi	^{137}Cs measured: _____ μCi	Does measured radioactivity fall within acceptable range (± 5%) for both ^{137}Cs and ^{57}Co?	Y / N
^{57}Co source reference ID: _____ _____μCi on _____ Expected radioactivity _____μCi Acceptable Range: _____μCi to _____μCi	^{57}Co measured: _____ μCi		
Verification of Analytical HPLC system			
Calibration curve date: _____ Slope (area/μmol): _____ Mass injected: _____ Expected peak area: _____ Acceptable range: _____ to _____	Measured peak area: _____ t_R: _____ min	Peak area within ± 10% of expected peak area?	Y / N

Post-Production Measurements and Release Tests	
Volume	**Data**
Subtract the weight of the empty dose vial from the full dose vial. Assume the density of the final product is approximately 1 g/mL to determine volume. Weight of full vial: _____ g; Weight of empty vial: _____ g	Volume of *[^{11}C]ER176 Injection*: _____ mL
Yield	
Measure the activity in the dose vial after removal of the QC sample.	Measured activity: _____ mCi at _____ (EOS)

Test	Test Data	Acceptance Criteria	Criteria Met
pH			
Dispense one to two drops of *[^{11}C]ER176 Injection* on pH paper.	Measured pH: _____	pH measured between 4.0 to 7.5?	Y / N
Membrane Filter Integrity			
Attach the syringe filter and needle used for sterile filtration to the compressed air source. Submerge needle tip in water. Pressurize to 45 psi.	Observation: Bubbles / No Bubbles	No bubbles observed from the submerged needle tip at 45 psi?	Y / N

[^{11}C]ER176 Injection: Quality Control Record

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Date of review: 2/18/2014

Appearance			
Visually inspect the *[^{11}C]ER176 Injection* in the dose vial.	Evaluate appearance	Colorless solution free of particulates?	**Y / N**
Radionuclidic Identity			
Determine the half life experimentally from two time points separated by at least three minutes.	t_1_____mCi at _____ t_2_____mCi at _____ Calc. half life: _____min	Experimental half life 20.4 ± 2 min?	**Y / N**
Residual Solvent			
Determine the residual ethanol and acetonitrile in dose by gas chromatography	Ethanol: _____ ng/µL Acetonitrile: _____ ng/µL Max. injectable vol: _____ mL Acetonitrile in inj. vol: _____ ng	Ethanol ≤ $1x10^5$ ng/µL? Acetonitrile ≤ 4.1×10^6 ng?	**Y / N** **Y / N**
Tests Based on HPLC and LC/MS Analysis[1]			
Measure the radioactivity of a 100 µL aliquot of *[^{11}C]ER176 Injection:* _____µCi @ _____ Measure background radiation before injection: _____µCi			
Radiochemical Identity			
Compare the retention time of *[^{11}C]ER176 Injection* to the ER176 standard retention time.	Standard t_R: _____ min Product t_R: _____ min	Difference is less than 1.0 min?	**Y / N**
Radiochemical Purity			
Integrate the peaks in the HPLC Bioscan trace. Determine the percent area represented by the product peak.	% Area: _____%	Greater than 95%?	**Y / N**
Chemical Purity			
Calculate the concentration of carrier and ER176 equivalent impurity in the *[^{11}C]ER176 Injection* from the peak area at 235 nm.	Carrier: _____ µg/mL Impurity: _____ µg/mL	Maximum volume contains no more than 5 µg of carrier or 0.5 µg ER176 equivalent impurity.	**Y / N**
Calculate the maximum injection volume as the lesser volume by maximum allowable carrier or maximum allowable equivalent impurity.	Max volume by carrier: _____ mL Max volume by impurity: _____ mL		
Specific Radioactivity			
Calculate the specific activity of *[^{11}C]ER176 Injection* in units of mCi/µmol	Calculated Specific Activity _____ mCi/µmol	Specific activity ≥ 500 mCi/µmol?	**Y / N**

[1] A calculations worksheet is typically used to perform the calculations required. A copy of the worksheet may be found in Appendix B.

[^{11}C]ER176 Injection: Quality Control Record

PET Radiopharmaceutical Sciences Section,
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Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 2/18/2014

Bacterial Endotoxin Test (Refer to SOP #QA302)

Reagents/Supplies	Lot # / Expiration Date
EndoSafe Unit ID	
Test Cartridge Lot Number	/
Sterile Water Lot Number	/
Pipette Tip Lot	/

	Result	Criteria	Acceptance Criteria Met?
Sample	_____ EU/mL Max injectable vol: _____mL EU in injectable vol.:_____	≤ 175 EU in total injectable volume	**Y / N**
Sample %CV		≤ 25%	**Y / N**
Spike		Refer to certificate of analysis of each cartridge lot for theoretical value	**NA**
Spike %CV		≤ 25%	**Y / N**
Recovery		50 – 200%	**Y / N**

All Quality Control specifications met?		**Y / N**
Chemist:	Signature:	Date:

[^{11}C]ER176 Injection: Post-Release Test Record

PET Radiopharmaceutical Sciences Section,
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National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Approved by: V. W. Pike Initials: V WP Date: 08/28/12
Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Batch # ER176-_____ Date: _____

1. Completed vial label
Attach completed vial label to this form.

2. Sterility (Reference SOP# QA302)

Procedure:

After radioactivity has decayed, place the formulated dose in the laminar flow cabinet with one Anaerobic (yellow-cap) and one Aerobic (blue-cap) Bactec test vial. Using aseptic technique, transfer approximately 100 µL formulated dose to each Bactec vial. Record the lot numbers and expiration dates on the sample submission form and take samples and form to the NIH Clinical Center Microbiology Lab.

Results:

Sample form should be returned by Microbiology Lab with test results within 2-4 weeks from date of submission. File results with this form.

Is the *[^{11}C]ER176 Injection* negative for aerobic and anaerobic growth? **Circle one: Yes or No**

Chemist:	Signature:	Date:

[^{11}C]ER176 Injection: Preparation of ER176 Standard Solution and HPLC Calibration Curve

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: 08/01/2012

Approved by: V. W. Pike Initials: VWP Date: 08/08/12

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

A. Preparation of ER176 Stock Solution

Refer to detailed procedures in SOP # QA305.

Procedure	Data/Results
Use an analytical balance with precision of at least 0.02 mg.	Balance ID / Location:
Record weight of 1 mg and 10 mg NIST traceable standards	1 mg weight: _____ 10 mg weight: _____
Are weights within ± 5% of expected values?	Y / N
Lot number of ER176 reference	
Lot number of acetonitrile (anhydrous)	
Mass of ER176 reference weighed into 25-mL volumetric flask	
Calculated concentration of ER176 reference stock solution	ng/ µL
Label solution as "ER176 Stock" with concentration and date of preparation. When not in use, solution should be stored at - 20 °C in a crimped sealed vial with butyl rubber septa.	

Chemist:	Signature:	Date:

[^{11}C]ER176 Injection: Preparation of ER176 Standard Solution and HPLC Calibration Curve

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: 08/01/2012

B. Preparation of ER176 Reference Standard for HPLC

Procedure	Data/Results
Date stock solution prepared	
Lot number of acetonitrile (anhydrous)	
Dispense 200 µL of the stock solution into a clean 10 mL volumetric flask. Dilute to the mark with acetonitrile.	
Calculated concentration of the diluted standard	ng/ µL
Label solution as "ER176 for HPLC" with concentration and date of preparation. When not in use, solution should be stored at - 20 °C in a crimped sealed vial with butyl rubber septa.	

Chemist Name:	Signature:	Date:

C. Generation of Calibration Curve

Procedure	Data/Results
Date standard solution prepared	
Inject the ER176 standard at four different volumes with a replicate of at least five injections each. Report the percent relative standard deviation for each replicate set (should be no more than 3%).	Level 1: _____µmol; %RSD: _____
	Level 2: _____ µmol; %RSD: _____
	Level 3: _____µmol; %RSD: _____
	Level 4: _____µmol; %RSD: _____
	All %RSDs below 3%? Y / N
Generate a calibration curve using a linear fit thorough the four injection points and the origin. Report the slope of the curve and the correlation coefficient (r^2). The correlation coefficient should be > 0.98.	Slope (area/µmol): r^2:
Attach the calibration curve plot and supporting chromatograms to this form. Edit the calculations worksheet to reflect the new calibration curve slope.	

Chemist Name:	Signature:	Date:

[^{11}C]ER176 Injection: Precursor and Reference Standard Acceptance Form

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

Date of review: 08/01/2012

Approved by: V. W. Pike Initials: VWP Date: 08/08/12

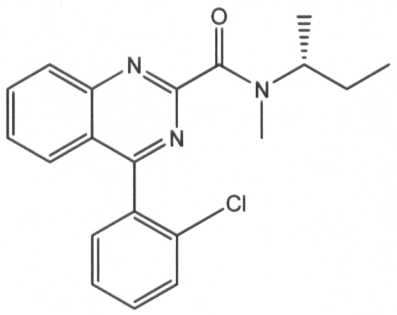
Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Initial Acceptance or Re-qualification (circle one) Date: _____

A. REFRENCE COMPOUND

1. Receipt of Compound:

Check the compound received and the supplier. Use a separate form for each compound.

ER176 Reference Standard (R)-N-sec-butyl-4-(2-chlorophenyl)-N-methylquinazoline-2-carboxamide	
□ Dip. Scienze Farmaceutiche-Università di Pisa, Via Bonanno 6, 56126 Pisa □ Other (specify):	
Date received:	
Lot number:	
Amount received:	
Received by:	

2. Attached Documents, Acceptance Criteria and Test Results:

All documents below should be provided by the supplier of the material and demonstrate that the material meets the acceptance criteria. If any data is not supplied, test should be performed at the PRSS facility.

Document	Acceptance Criteria	From Supplier	From PRSS
Certificate of Analysis	N/A	**Y / N**	N/A
^{1}H-NMR	Consistent with structure	**Y / N**	**Y / N**
HPLC Chromatogram	Product is ≥ 95% of total peak area	**Y / N**	**Y / N**
LC-MS	Consistent with structure	**Y / N**	**Y / N**

File all supporting data with this form.

Material accepted for use in synthesis of *[^{11}C]ER176 Injection*? **Y / N**

Chemist:	Signature:	Date:

[^{11}C]ER176 Injection: Precursor and Reference Standard Acceptance Form

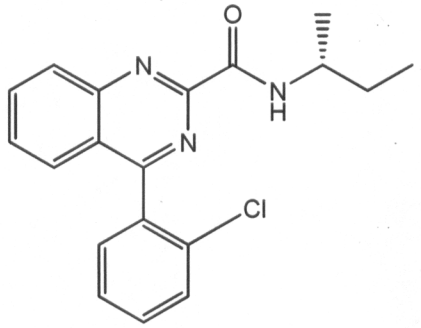
PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: 08/01/2012

B. PRECURSOR COMPOUND

1. Receipt of Compound:

Check the compound received and the supplier. Use a separate form for each compound.

ER176 Precursor (R)-N-sec-butyl-4-(2-chlorophenyl)quinazoline-2-carboxamide	
□ Dip. Scienze Farmaceutiche-Università di Pisa, Via Bonanno 6, 56126 Pisa □ Other (specify):	
Date received:	
Lot number:	
Amount received:	
Received by:	

2. Attached Documents, Acceptance Criteria and Test Results:

All documents below should be provided by the supplier of the material and demonstrate that the material meets the acceptance criteria. If any data is not supplied, test should be performed at the PRSS facility.

Document	Acceptance Criteria	From Supplier	From PRSS
Certificate of Analysis	N/A	**Y / N**	N/A
^{1}H-NMR	Consistent with structure	**Y / N**	**Y / N**
HPLC Chromatogram	Product is ≥ 95% of total peak area	**Y / N**	**Y / N**
LC-MS	Consistent with structure	**Y / N**	**Y / N**

File all supporting data with this form.

Material accepted for use in synthesis of *[^{11}C]ER176 Injection*? **Y / N**

Chemist:	Signature:	Date:

[^{11}C]ER176 Injection: Certificates of Analysis

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: 8/28/2012

Certificates of Analysis
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PET Radiopharmaceutical Sciences Section,
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National Institutes of Health,
Bldg. 10, Rm. B3 C338,
Bethesda, MD 20892

Date of review: 8/28/2012

Dimethyl sulfoxide, anhydrous

Certificate of Analysis

SIGMA-ALDRICH

Product Name	Dimethyl sulfoxide, anhydrous, ?99.9%
Product Number	276855
Product Brand	SIAL
CAS Number	67-68-5
Molecular Formula	$(CH_3)_2SO$
Molecular Weight	78.13

TEST	SPECIFICATION	LOT 01444DE RESULTS
APPEARANCE	COLORLESS LIQUID	COLORLESS LIQUID
INFRARED SPECTRUM		CONFORMS TO STRUCTURE.
GAS LIQUID CHROMATOGRAPHY	99.90% (MINIMUM)	99.99%
TITRATION	0.005% H2O (MAXIMUM)	0.0028% H2O
RESIDUE ON EVAPORATION	0.0005% (MAXIMUM)	0.00017%
QUALITY CONTROL ACCEPTANCE DATE		MAY 2006

Barbara Rajzer

Barbara Rajzer, Supervisor
Quality Control
Milwaukee, Wisconsin USA

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: 8/28/2012

Potassium hydroxide, 85%

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Potassium hydroxide, ACS reagent, ? 85%, pellets
Product Number	221473
Product Brand	Sigma-Aldrich
CAS Number	1310-58-3
Molecular Formula	KOH
Molecular Weight	56.11

TEST	SPECIFICATION	LOT 05611JD RESULTS
APPEARANCE	WHITE PELLETS	WHITE PELLETS
TITRATION	85.0% KOH (MINIMUM) (WITH HCL)	89.73% KOH (WITH HCL) *
	2.0% K2CO3 (MAXIMUM)	0.50% K2CO3 *
ICP ASSAY	CONFIRMS POTASSIUM COMPONENT.	CONFIRMS POTASSIUM COMPONENT
CALCIUM	0.005% (MAXIMUM)	<0.001% *
CHLORIDE	0.01% (MAXIMUM)	<0.005% *
IRON	0.001% (MAXIMUM)	<0.0005% *
HEAVY METALS	0.001% (MAXIMUM) (AS AG)	<0.001% (AS AG) *
MAGNESIUM	0.002% (MAXIMUM)	<0.001% *
SODIUM	0.05% (MAXIMUM)	<0.01% *
NITROGEN COMPOUNDS	0.001% (MAXIMUM)	<0.001% *
NICKEL	0.001% (MAXIMUM)	0.0005% *
PHOSPHATE	5 PPM (MAXIMUM)	<3 PPM *
SULFATE	0.003% (MAXIMUM)	<0.001% *
	AND/OR	* SUPPLIER DATA
	MATERIAL TYPICALLY CONTAINS 10%-15% H2O	* SUPPLIER DATA
	REVISED DECEMBER 17, 2003 JSB	* SUPPLIER DATA
	AND/OR	MEETS REQUIREMENTS OF ACS 9TH ED
	MATERIAL TYPICALLY CONTAINS 10%-15% H2O	MEETS REQUIREMENTS OF ACS 9TH ED
	REVISED DECEMBER 17, 2003 JSB	MEETS REQUIREMENTS OF ACS 9TH ED
RECOMMENDED RETEST		JULY 2007

PET Radiopharmaceutical Sciences Section,
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Bethesda, MD 20892

Date of review: 8/28/2012

PRODUCT CROSS EXACT REPLACEMENT FOR PRODUCT NUMBERS P6310

REFERENCE INFORMATION

QUALITY CONTROL

ACCEPTANCE DATE JULY 2005

Barbara Rajzer, Supervisor
Quality Control
Milwaukee, Wisconsin USA

Ethyl alcohol, USP 200 proof

MGP INGREDIENTS, INC.
1300 Main Street, P.O. Box 130 • Atchison, Kansas 66002-0130
913.367.1480 • 1.800.255.0302 • Fax 913.367.0192 • www.mgpingredients.com

CERTIFICATE OF ANALYSIS
USP ETHANOL, 200 PROOF
GRAIN ALCOHOL

MGPI LOT#:	**10-100730-000219**
PO#3915-*00	
CARRIER #:	**DBUX 300785**
MANUFACTURING DATE:	**06-11-07**
SHIPPING DATE:	**06-11-07**
CUSTOMER:	**WARNER GRAHAM**

PROPERTY	METHOD	ANALYSIS	SPECIFICATION
SPECIFIC GRAVITY @ 15.56°C, in air	Mettler Density Meter	0.79371	Not more than 0.7962
PERCENT MOISTURE BY WEIGHT	Karl Fischer titration	0.03	0.1% Maximum
IR SCAN	USP	97.77	Matches USP reference std.
CLARITY	USP	99.33	Passes USP test for clarity
COLOR	USP	99.26	Passes USP test for color
ALKALINITY	USP	Passes Test	Colorless
ACIDITY	USP	Passes Test	< 30 ppm, as acetic acid
ULTRAVIOLET ABSORPTION:	USP		
From 200 to 400 nm (against air):			
• @ 240 nm		0.318	0.40 maximum
• @ 250 nm		0.168	0.30 maximum
• @ 260 nm		0.096	0.30 maximum
• between 270 and 340 nm		0.033	0.10 maximum
Curve between 235 and 340 nm (against water)		Smooth	The absorption curve is smooth
VOLATILE IMPURITIES:	GC		
Acetaldehyde and/or Acetal		7.83	Not more than 10 ppm
Methanol		15.59	Not more than 200 ppm
Benzene		ND*	Not more than 2 ppm
Other Impurities		None Reportable	Not more than 300 ppm
LIMIT OF NONVOLATILE RESIDUE	USP	0.1 mg	Not more than 2.5 mg/100mls
ODOR	Organoleptic	Passes	Clean, Neutral

This material conforms to the current USP monograph for "Dehydrated Alcohol".

LOT# MGPO7176RR

WG Lot# 254712

Jennifer Peltzer, QA Lab Manager

Form #:	Dept.:	Issued:	Revision:	Approved:
USP 200 - Warner Grahams	QA LAB	03-08-07	New	J. Peltzer

PET Radiopharmaceutical Sciences Section,
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Bethesda, MD 20892

Date of review: 8/28/2012

0.9% Sodium Chloride for Injection, USP

APP PHARMACEUTICALS
3159 Staley Road, Grand Island, NY 14072, (716) 773-0800

Certificate of Analysis

Finished Product

Product Name:	Sodium Chloride Injection, USP		
Product Code:	918610	Lot Number:	408021
Manufacture Date:	9/16/09		
Release Date:	10/10/09	Expiration Date:	09/11

Analysis	Specifications	Results	Conforms
Assay (9mg/mL)	95.0 – 105.0% of Label Claim	99.9%	✓
Color of Solution	Compares to Standard	Compares to Standard, 0	✓
Heavy Metals	NMT 0.001%	NMT 0.001%	✓
Identification 1. Chloride 2. Sodium	 1. Solutions of chlorides yield with silver nitrate TS a white, curdy precipitate that is insoluble in nitric acid but is soluble in a slight excess of 6 N ammonium hydroxide. 2. Add 15% potassium carbonate, no precipitate is formed. Add potassium pyroantimonate TS, a dense precipitate is formed. Sodium compounds impart an intense yellow color to a non-luminous flame.	 1. Meets Specification 2. Meets Specification	 ✓ ✓
Iron	NMT 2 ppm	NMT 2 ppm	✓
Labeling	The label states the total osmolar concentration in mOsmol per liter.	Meets Specification	✓
pH	4.5 – 7.0	5.1	✓
Visual Inspection 1. Container/Closure 2. Color/Clarity	 1. Container is intact 2. Clear, no Particulate Matter present	 1. Meets Specification 2. Meets Specification	 ✓ ✓
Volume in Container 10 mL Label Claim	NLT Label Claim	Meets Specification	✓
Other Requirements	Meets the requirements under Injections USP <1>	Meets USP requirements	
Sterility	Sterile	Sterile	✓
Particulate Matter	1. Does not exceed 6000 particles ≥ 10 microns per container 2. Does not exceed 600 particles ≥ 25 microns per container	1. 39 2. 1	✓ ✓
Bacterial Endotoxins	NMT 0.5 EU/mL	<0.3 EU/mL	✓

This product conforms to the specifications established by APP Pharmaceuticals

12/10/09
Product Release Management

11/23/09

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: 8/28/2012

Ethanol, dehydrated

LUITPOLD

CERTIFICATE OF ANALYSIS

PRODUCT: Dehydrated Alcohol Injection, USP

LOT NUMBER: 9664

CODE NUMBER: 8571

FILL SIZE: 1 mL ampule

CONCENTRATION: 99.5%

EXPIRATION DATE: SEP11

DATE OF MANUFACTURE: 09/29/09

REVIEWED BY:
Lisa A Meigel 2/19/10

APPROVED BY:
Ginamarie Currao 2-19-10
Ginamarie Currao
Quality Assurance Supervisor
Luitpold Pharmaceuticals, Inc.

Page 1 of 2

TEST	SPECIFICATIONS	RESULTS
Description:	Clear, colorless, mobile, volatile liquid having a characteristic odor.	Passes
Identification:	A. Intense blue color is produced.	Passes
	B. Odor of iodoform develops and a yellow precipitate is formed.	Passes
Specific Gravity:	<841> Not more than 0.8035 at 15.56°C indicating not less than 96.8% by weight of C_2H_5OH.	Passes
Acidity:	Not more than 10.0 mL of 0.020 N NaOH is required for neutralization.	Passes
Limit of Non-volatile Residue:	Limit is ≤ 1 mg.	Passes
Water-Insoluble Substances:	Mixture is clear.	Passes
Aldehydes & other foreign organic substances:	The pink color does not entirely disappear.	Passes

LUITPOLD PHARMACEUTICALS, INC. ·ONE LUITPOLD DRIVE·SHIRLEY·NEW YORK 11967
631-924-4000·FAX: 631-924-1731

2/22/10

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: 8/28/2012

LUITPOLD

CERTIFICATE OF ANALYSIS

PRODUCT: Dehydrated Alcohol Injection, USP

LOT NUMBER: 9664

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REVIEWED BY:
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APPROVED BY:
Ginamarie Currao 2-19-10
Ginamarie Currao
Quality Assurance Supervisor
Luitpold Pharmaceuticals, Inc.

Page 2 of 2

TEST	SPECIFICATIONS	RESULTS
Amyl Alcohol & Nonvolatile Carbonizable Substances:	No red or brown color is produced.	Passes
Ultraviolet Absorbance:	Limit is not more than 0.08 at 240 nm and 0.02 between 270 nm and 340 nm.	Passes
Limit of Acetone & Isopropyl Alcohol:	Any pink color produced in the test solution is not more intense than that in the control.	Passes
Methanol:	No violet color appears.	Passes
Volume Check:	Between 1.0 mL and 1.3 mL.	Passes
Sterility:	<71> If no growth is observed, the article tested meets the requirements of the test for sterility.	Passes
Particulate Matter:	<788> Light Obscuration Particle Count Test: Limit is 6,000 particles per container that are ≥ 10 μm. Limit is 600 particles per container that are ≥ 25 μm.	Passes Passes

This product manufactured in the USA complies with all USP testing requirements for release of the finished product.

LUITPOLD PHARMACEUTICALS, INC. ·ONE LUITPOLD DRIVE·SHIRLEY·NEW YORK 11967
631-924-4000·FAX: 631-924-1731

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: 8/28/2012

LUITPOLD

CERTIFICATE OF COMPLIANCE

PRODUCT: Dehydrated Alcohol Injection, USP

LOT NUMBER: 9664

CODE NUMBER: 8571

FILL SIZE: 1 mL ampule

CONCENTRATION: 99.5%

EXPIRATION DATE: SEP11

DATE OF MANUFACTURE: 09/29/09

REVIEWED BY:
Lisa A Meigel 2/19/10

APPROVED BY:
Ginamarie Currao 2-19-10
Ginamarie Currao
Quality Assurance Supervisor
Luitpold Pharmaceuticals, Inc.

To Whom It May Concern:

This document certifies that the above mentioned lot meets all testing specifications, meets the requirements under Injections <1>, and has been manufactured in compliance with Current Good Manufacturing Practice and our established Standard Operating Procedures. This product was manufactured in the USA.

LUITPOLD PHARMACEUTICALS, INC. ·ONE LUITPOLD DRIVE·SHIRLEY·NEW YORK 11967
631-924-4000·FAX: 631-924-1731

Sterile vial 10 mL

HOSPIRA, INC. - ROCKY MOUNT, NC

DATED: 09-04-20 W5816BQAC PAGE 1

REVW *07-01-22* EFFC *09-04-20* SUBTYPE-8 AREA-B
Desc: Certificate of Analysis Hospira, Inc. (Rocky Mount N.C.) BQ
Written By/Date: B. Whitehead 1-22-07 Unit: SVP/Rocky Mount
Specification Comparison Completed By/Date: BQ L. Oliver 1-22-07
MQ J. Fisher 1-26-07

Specification No.: 61.05816ALLCODE, QPO.18.004

Technical Note:
1. This product has been manufactured and tested in current good manufacturing practices (CGMP) facilities in accordance with appropriate regulations. This product meets applicable specifications, applicable regulatory submissions or marketing authorizations and, where appropriate, compendial requirements. The undersigned certifies this to be a true representation of the results.

Product Manufacturing Information

Date of Manufacture: 08-24-09 Batch Size N/A NDC/DIN No.: N/A

STEP/PROCEDURE	:DOC. REF.	:SIGNATURE/DATE
Manufacturing Formula	:92.D-N/A :Current Date: N/A	:
Commodity and Process Summary	:35. 05816 0911 :Current Date: 07-30-07	:
Printed Material Summary	:40. 05816 04AC :Current Date: 04-18-07	:
Sampling and Testing Requirements	:61.05816ALLCODE :Current Date: 06-12-89	: 09-09-09

11/20/09

5816-04-11 10 ML 80-562-EV AGE LIMIT 1AUG2013 JD13213
STERILE EMPTY VIAL

COMMENTS:

ISSUER: MCQUEJT 08/24/09

HOSPIRA, INC. - ROCKY MOUNT, NC

DATED: 09-04-20 W5816BQAC PAGE 2

PRODUCT TEST RESULTS

STEP/PROCEDURE	DOC. REF.	RESULTS	SIGNATURE/DATE
I. Physical Requirements			
A. Visual	90.P-0146	Parsley	
1. Sealed sterile container (bottle) must have no visible opening or defect which would permit biological contamination, (ie no cracks, splits, checks, crizzles or other opening in the glass bottle, and the stopper completely inserted and held in place by the overseal.		Pass ✓ Fail___	
2. The container and closure components must be correct as specified and listed in the mps summary.			
3. Overseal must be complete, unbroken, and in proper position.			
4. There must be no checks or cracks greater than 20% through the glass wall as judged by an inspector.			
5. Sterile sealed container and overwrap (where applicable) must be free of obvious soil on outside surfaces, or other defects detracting from good appearance or workmanship.			

5816-04-11 10 ML 80-562-EV AGE LIMIT 1AUG2013 JD13213
STERILE EMPTY VIAL

COMMENTS:

ISSUER: MCQUEJT 08/24/09

HOSPIRA, INC. - ROCKY MOUNT, NC

DATED: 09-04-20 W5816BQAC PAGE 3

STEP/PROCEDURE	DOC. REF.	RESULTS	SIGNATURE/DATE
II. Biological Requirements:			
A. Bacterial Endotoxin Not more than 0.25 EU/ML	90.B-0756	< 0.03 EU/ML Pass ✓ Fail ___	BCull 8/28/09
B. Environmental Data	QPO.29.002		
1. Must meet requirements	WGENVIRO WGENVISO	Pass ✓ Fail ___	BCull 8/29/09
B. Sterility			
1. Must meet test requirements	90.M-0229	Pass ✓ Fail ___	BCull 9/9/09
			BCull 9/9/09 BQ REVIEW
			09.09.09 BRQ

END OF DOCUMENT

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: 8/28/2012

Sterile Millex-GV filter (0.22 µm, 4 mm diameter)

MILLIPORE

Certificate of Quality

Item Description: Millex 4mm Durapore PVDF .22um Sterile 100/pk

Millipore Catalog Number: SLGV004SL

Millipore Lot Number: R9BN60078

Millipore Corporation certifies that the above-referenced product:

- Was manufactured (or supplied) by a Millipore facility whose Quality Management System is approved by an accredited registering body to the appropriate ISO 9001 Quality Systems Standard.
- Was manufactured in accordance with applicable Millipore Standard Procedures.
- Was tested in accordance with applicable Millipore Quality Specifications.

Michelle Coll

Michelle Coll
Quality Assurance

Date Verified: 20-May-2009

The format of this document has been updated to include approval date , page numbers and/or print date.

Sterile Millex-MP filter (0.22 µm, 25 mm diameter)

Sterile Millex® Filter Unit

Pore Size Rating: 0.22 µm
Catalogue Number: SLMPL25SS
Lot Number: R9JN19014
Expiry Date: 2012 07
Sterilization Date: 2009 07
Membrane Type: Hydrophilic TCMF, PES

Good Manufacturing Practice

This product was manufactured in a Millipore Facility that meets FDA Device Good Manufacturing Practice Standards under the Quality System Regulation and ISO 13485 Standard for Medical Device production.

CE Marking

Product is CE marked in accordance with EC directive 93/42EEC 1993

ISO 9001 Quality Standard

This product was manufactured in a Millipore facility whose Quality Management System is approved by an accredited registering body to the ISO 9001 Quality Systems Standard.s Standard.

Component Materials Toxicity

Component materials were tested and meet the criteria for the current USP Biological Test for Plastics.

Millex® is a registered trademark of Millipore Corporation or an affiliated company.

Rev 04/06

Quality Assurance Lot Release Criteria

This manufacturing lot was sampled, tested and released by Quality Assurance to the following specification:

Bacterial Endotoxins

An aqueous extraction from the unit contains less than or equal to 2.15 EU/Unit as determined using the Limulus Amebocyte Lysate (LAL) Test.

Integrity

Each unit is air tested during the manufacturing process to ensure both membrane and housing integrity. Prior to release, samples are tested to meet a water bubble point specification of ≥ 56psi and particle challenge tested by a method that correlates to the Brevundimonas diminuta HIMA bacterial challenge test.

Housing Burst

Samples meet a minimum housing burst of 75 psi

Water Flow Rate

Samples exhibit a water flow rate greater than or equal to 175ml per minute at 30 psi with 0.22µm filtered RO water at 25ºC

Sterility

This product has been sterilized by Ethylene Oxide (EO) in a validated sterilization cycle according to ISO 11135 standard. Spore strips (Bacillus atrophaeus 10^6) incorporated in the lot have been shown to be sterile.

Maximum EO residue limits meet the U.S. Federal guideline for devices contacting blood (ex vivo).

Quality Assurance Audit Criteria

This product was designed and manufactured to meet the following characteristics that are confirmed by testing on an audit basis.

Toxicity

Non-toxic as per the current USP Mouse Safety Test.

Downstream Particles

Samples show no more than 50 particles >10 µm per unit.

Joe Bergin

QA Manager - Millipore Ireland B.V.

Carrigtwohill
Co. Cork
Ireland

MILLIPORE

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: 8/28/2012

Sterile needle (21 G, 2")

1 Becton Drive
Franklin Lakes, NJ 07417
tel: 201.847.6800
www.bd.com

CERTIFICATE OF QUALITY

BD

BD Reorder Number: 305129 **Lot Number:** 9328139

US FDA Quality System Regulation (GMP) Compliance -- BD Products are manufactured in accordance with the current FDA Quality System Regulation.

Quality Control Testing - Representative production samples are collected and inspected in accordance with current applicable product specifications. Inspection records are reviewed and signed off by qualified personnel for product release.

Toxicity – All materials which are labeled non-toxic and released for sale by BD have passed animal toxicity and or cytoxicity in accordance with ISO 10993 Biocompatibility Testing Guidelines for Medical Devices.

Products Labeled Non-Pyrogenic – All products which are labeled as non-pyrogenic and released for sale by BD meet BD pyrogen testing requirements.

Sterilization – All products which are labeled as sterile and released for sale by BD are certified to be sterile as long as the package is unopened and undamaged. For those products labeled "sterile fluid path", only the fluid path is sterile. Sterilization cycle development/validation is performed in accordance with current ANSI/AAMI/ISO guidelines.

Device Listing/Manufacturing Site Registration: Medical devices are listed with FDA per 21CFR807. Manufacturing sites are registered with FDA per 21CFR807.

VERIFICATION:

K Alderman

Keith Alderman
VP Quality
Medical Surgical Systems

Becton, Dickinson and Company

Sterile needle (20 G; 1.5")

1 Becton Drive
Franklin Lakes, NJ 07417
tel: 201.847.5600
www.bd.com

CERTIFICATE OF QUALITY

BD

BD Reorder Number: 305176 **Lot Number: 9119619**

US FDA Quality System Regulation (GMP) Compliance – BD Products are manufactured in accordance with the current FDA Quality System Regulation 21CFR820.

Quality Control Testing - Representative production samples are collected and inspected in accordance with current applicable product specifications. Inspection records are reviewed and signed off by qualified personnel for product release.

Toxicity – All materials which are labeled non-toxic and released for sale by BD have passed animal toxicity and or cytoxicity in accordance with ISO 10993 Biocompatibility Testing Guidelines for Medical Devices.

Products Labeled Non-Pyrogenic – All products which are labeled as non-pyrogenic and released for sale by BD meet BD pyrogen testing requirements.

Sterilization – All products which are labeled as sterile and released for sale by BD are certified to be sterile as long as the package is unopened and undamaged. For those products labeled "sterile fluid path", only the fluid path is sterile. Sterilization cycle development/validation is performed in accordance with current ANSI/AAMI/ISO guidelines.

Device Listing/Manufacturing Site Registration/Pre-Market Notification: Medical devices are listed with FDA per 21CFR807. Manufacturing sites are registered with FDA per 21CFR807. The devices satisfy FDA pre-market notification requirements per 21CFR 807.

VERIFICATION:

K Alderman

Keith Alderman
VP Quality
Medical Surgical Systems

Methanol, HPLC grade

Certificate of Analysis

Page 1 of 1

Fisher Scientific

1 Reagent Lane
Fairlawn, NJ 07410
201.796.7100 tel
201.796.1329 fax

Certificate of Analysis

Fisher Scientific's Quality System has been found to conform to Quality Management System Standard ISO9001:2000 standard by DNV Certificate number CERT-08052-2006-AQ-HOU-ANAB

This is to certify that units of the above mentioned lot number were tested and found to comply with the specifications of the grade listed. Certain data have been supplied by third parties. Fisher Scientific expressly disclaims all warranties, expressed or implied, including the implied warranties of merchantability and fitness for a particular purpose. Certain products (USP/FCC/NF/EP/BP/JP grades) are sold for use in food, drug, or medical device manufacturing. Fisher does not claim regulatory coverage under 21 CFR nor maintain DMF's with the FDA. The following are the actual analytical results obtained:

Catalog Number	**A452**	**Mfg. Date**	**2/5/2010**
Lot Number	096964		
Description	**METHANOL, HPLC GRADE, A.C.S.**		
Country of Origin	**Trinidad**		
Chemical Origin	**Organic - synthetic**		
BSE/TSE Comment	No animal products are used as starting raw material ingredients, or used in processing, including lubricants, processing aids, or any other material that might migrate to the finished product.		

Result name	Units	Specifications	Test Value
APPEARANCE		REPORT	CLEAR, COLORLESS LIQUID
ASSAY	%	>= 99.9	99.9
CARBONYL COMPOUNDS	%	<= 0.001	0.0006
COLOR	APHA	<= 5	5
EVAPORATION RESIDUE	ppm	<= 3	0.5
FLUORESCENCE BACKGRD	PASS/FAIL	= PASS TEST	PASS TEST
IDENTIFICATION	PASS/FAIL	= PASS TEST	PASS TEST
LC GRADIENT SUITABLE	PASS/FAIL	= PASS TEST	PASS TEST
OPTICAL ABS AT 205 NM	ABSORBANCE UNITS	<= 1.00	0.87
OPTICAL ABS AT 220 NM	ABSORBANCE UNITS	<= 0.30	0.23
OPTICAL ABS AT 230 NM	ABSORBANCE UNITS	<= 0.15	0.10
OPTICAL ABS AT 254 NM	ABSORBANCE UNITS	<= 0.025	0.010
REF. INDEX AT 25 DEG. C		Inclusive Between 1.3260 1.3300	1.3275
SOLUBILITY IN WATER	PASS/FAIL	= PASS TEST	PASS TEST
SUBSTANCES DARKENED BY H2SO4	PASS/FAIL	= PASS TEST	PASS TEST
SUBSTANCES REDUCING KMNO4	PASS/FAIL	= PASS TEST	PASS TEST
TITRATABLE ACID	mEq/g	<= 0.0003	0.00020
TITRATABLE BASE	mEq/g	<= 0.0002	0.00004
WATER (H2O)	%	<= 0.1	0.06

CERTIFIED BY | Joel Boland

Lab Manager BPF

Note: The data listed is valid for all package sizes of this lot of this product, expressed as a extension of this catalog number listed above. If there are any questions with this certificate, please call Chemical Services at (800) 227-6701.

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: 8/28/2012

Water, HPLC grade

EMD Chemicals Inc.
480 S. Democrat Road
Gibbstown, NJ 08027
Phone 856–423–6300
Fax 856–423–4389

Name: Water
HPLC Grade

Formula: H_2O

Item Number: WX0008–1, WX0008–901

Formula Wt: 18.02

Lot Number: 49273

Data Order No: 000421580

CHARACTERISTIC	REQUIREMENT		RESULTS	UNITS
	Min.	Max.		
#Expiration date			1–SEP–2010	
Appearance			Clear liquid free from particulates	
Color (APHA)		10	< 10	
Filtered through 0.2 µm filter			Passes test	
Fluorescence (as quinine base)		150	25	ppt
Gradient at 210 nm		0.002	0.00051	AU
Gradient at 254 nm		0.0005	0.00030	AU
Odor			None	
Refractive index (n 20/D)			1.3330	
Residue after evaporation		2	< 0.5	ppm
UV Abs. at 190 nm		0.01	0.007	AU
UV Abs. at 210 nm		0.01	0.005	AU
UV Abs. at 250 nm		0.005	0.002	AU
UV Abs. at 280 nm		0.005	0.002	AU

Charles M. Wilson,
Quality Control Manager

Release Date: 9/29/2009

EMD Chemicals Inc.
(Formerly EM Science, A Division of EM Industries, Inc.)

An Affiliate of Merck KGaA, Darmstadt, Germany

Acetonitrile, HPLC grade

4/29/2009

Honeywell

Honeywell Burdick & Jackson™
1953 South Harvey Street
Muskegon, MI 49442
Phone: (800) 368-0050
Fax: (231) 728-8226

Certificate of Analysis

Product:	Acetonitrile
Cat:	017
Lot:	CZ196
Date of Manufacture:	4/28/09
Use Before Date:	10/28/10

Low Water Content
Meets UV Specifications

Test	Specification Min.	Max.	Result	Units
Water by Karl Fischer Titration		0.003	0.001	%
UV Cutoff		190	189	nm
Refractive Index (20°C)	1.3434	1.3446	1.3439	
Residue		1	<0.5	mg/L
GC Analysis	99.9		>99.99	%
UV Absorbance @ 200 nm		0.050	0.0308	AU
UV Absorbance @ 210 nm		0.040	0.0285	AU
UV Absorbance @ 225 nm		0.010	0.0094	AU
UV Absorbance @ 250 nm		0.005	0.0019	AU
UV Absorbance @ 350 nm		0.005	0.0024	AU

9/23/09

Quality Control Approval

LIMS Sample No.: AC03375

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: 8/28/2012

Ammonium hydroxide, 1 N (aq)

RICCA CHEMICAL COMPANY

Arlington, TX 76012
Pocomoke City, MD 21851
Batesville, IN 47006
http://www.riccachemical.com
1-888-GO-RICCA
customerservice@riccachemical.com

Certificate of Analysis

Ammonium Hydroxide, 1.00 Normal

Lot Number: 2812347 Product Number: 642 Expiration Date: DEC 2009 Manufacture Date:12/11/2008

Contains:

Name	CAS#	Grade
Ammonium Hydroxide, NH4OH	1336-21-6	ACS
Water, Deionized, H2O	7732-18-5	ACS, ASTM D 1193 (Type I), EP, USP

Test Name	Assay Method	Specification	Result
Appearance	Clarity, Color, Odor	Clear, colorless, ammonia odor	Passed Test
Assay at 20 °C (traceable to NIST SRM 723)	Titrimetric vs. Sulfuric Acid (Methyl Red Indicator)	1.001 ± 0.001 N at 20.0 °C	1.000 N at 20.0 °C

Specification	Reference	Method Number
Ammonium Hydroxide, 1 N	EPA	130.2
Ammonium Hydroxide, 1 N	EPA	130.1

Volumetric glassware complies with Class A tolerance requirements of ASTM E 288 and NIST Circular 434; it is calibrated before first use and recalibrated regularly in accordance with ASTM E 542 and NIST Procedure NBSIR 74-461. Balances are calibrated regularly with weights certified traceable to the NIST national mass standard. Thermometers and temperature probes are calibrated before first use and recalibrated regularly with a thermometer traceable to NIST standards. All products are prepared according to master documents that assure manufacture according to validated methods. Batch records document raw material traceability and production and testing history for each lot manufactured.

Shelf Life (unopened container):

Part Number	Shelf Life
642-32	12 months
642-5	12 months
642-1	12 months
642-16	12 months

Recommended Storage: 15°C - 30°C (59°F - 86°F)

LaNelle Ohlhausen
Quality Assurance

This Certificate of Analysis is designed to comply with ISO Guide 31 "Reference Materials -- Contents of Certificates and Labels."
To determine manufacturing site using lot number, visit www.riccachemical.com/AboutUs/lot.pdf.

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: 8/28/2012

HPLC column (analytical, Phenomenex Luna; 10 µm; 4.6 mm × 250mm)

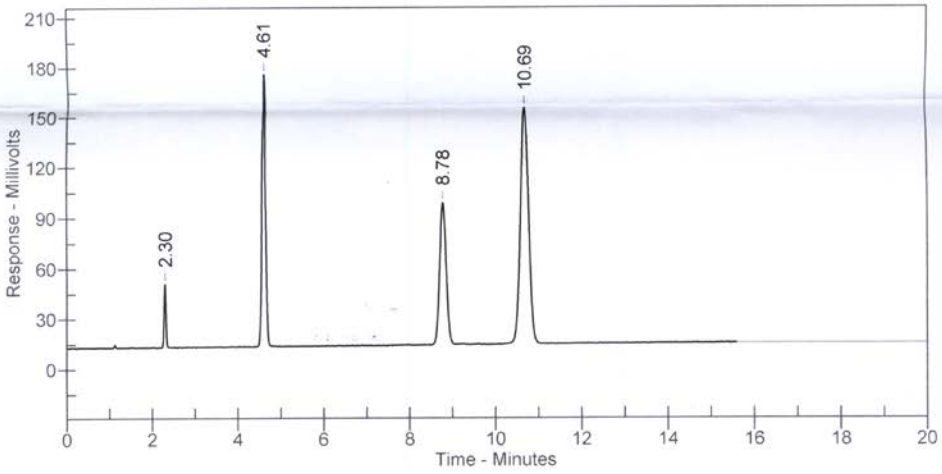
phenomenex
...breaking with tradition™

HPLC Certificate of Quality Assurance

Part No: 00G-4253-E0 **Serial No.: 600405-6**
Description: Luna 10u C18(2) 100A **Size:** 250 X 4.6 mm

Measured Parameters Based on the NAPHTHALENE Peak

Efficiency: 58756 plates/meter **k Factor:** 3.649 **Batch No:** 5293-60
Peak Asym.: 1.02 **Ret. Time:** 10.69 minutes



Test Analytes

Peak	Analyte	Time	Area	k Factor	Width	Eff.	Asym.
1	URACIL	2.30	116527	0.000	0.047	13185	1.10
2	ACETOPHENONE	4.61	928757	1.006	0.089	14917	1.05
3	TOLUENE	8.78	895149	2.820	0.166	15411	1.05
4	NAPHTHALENE	10.69	1866824	3.649	0.207	14689	1.02

Testing Condition

Mobile Phase: 65% MECN/H2O **Temperature:** AMBIENT
Flow Rate: 1 mL **Injection:** 1 µL
Detection: 254 nm **Back Pressure:** 769 psi

Checked By:
Approved By:

QA #4 NOV-1 2011 APPROVED

Instrument: LC-1100-31
Tested: Sunday, October 30, 2011
Printed: Sunday, October 30, 2011

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: 8/28/2012

HPLC column (semi-prep, Waters XTerra RP18; 10 µm; 7.8 mm × 300 mm)

Certificate of Analysis

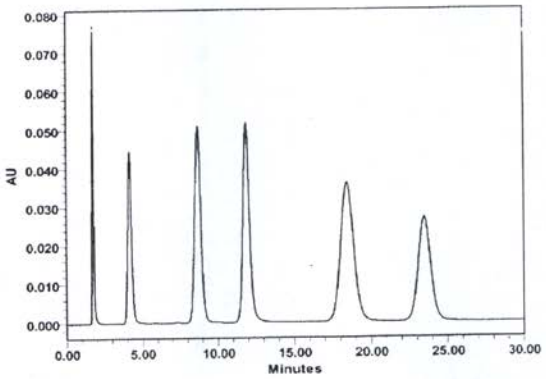
XTerra® Prep RP_{18}, 10 µm

Batch # 127I

Chromatographic Results* for XTerra® Prep RP_{18}, 10 µm

Peak Identification

1. Uracil
2. Propranolol
3. Butyl Paraben
4. Naphthalene
5. Amitriptyline
6. Acenaphthene



	Specification	Result
k Acenaphthene		12.37
Relative Retention (r)		
Propranolol/Acenaphthene	0.100 - 0.119	0.112
Butyl Paraben/Acenaphthene	0.299 - 0.343	0.319
Naphthalene/Acenaphthene	0.459 - 0.477	0.466
Amitriptyline/Acenaphthene	0.722 - 0.798	0.768
USP Tailing Factor		
Amitriptyline	≤ 1.40	1.05
Propranolol	≤ 1.45	1.10

**Chromatographic Conditions: Column: 4.6 mm x 150 mm, Flow Rate: 1 mL/min*
Mobile Phase: 40.0% v/v 20.0 mM KH_2PO_4/K_2HPO_4 at pH 7.00/ 60.0 % Methanol at 23°C

Material Approved: Jean P. Bachman
Quality Control

Date: 30 August 2011

Form # 1394 – Rev. 2

Waters

Waters Corporation 34 Maple Street Milford, Massachusetts 01757-3696 U.S.A. 508 478-2000 www.waters.com

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: 8/28/2012

Certificate of Analysis

XTerra® Prep RP_{18}, 10 µm

Batch # 127I

Analytical Results for XTerra® Prep RP_{18}, 10 µm

	Result
Analysis of Unbonded Particles	
10 µm Particle Size Distribution	
90 % / 10 % Diameter Ratio	2.22
Particle Consistency	Pass
Pore Structure	
Pore Volume	0.63 cm^3/g
Average Pore Diameter	133 Å
Surface Area	164 m^2/g
Metal Impurity Concentrations	
Na	<1 ppm
Al	<1 ppm
Fe	<1 ppm
Analysis of XTerra Prep RP_{18}, 10 µm	
Total Carbon Content	14.36 %
C_{18} Surface Concentration	2.35 µmoles/m^2

Material Approved: _____ Date: 30 August 2011
Quality Control

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: 8/28/2012

XTerra® PREP COLUMNS

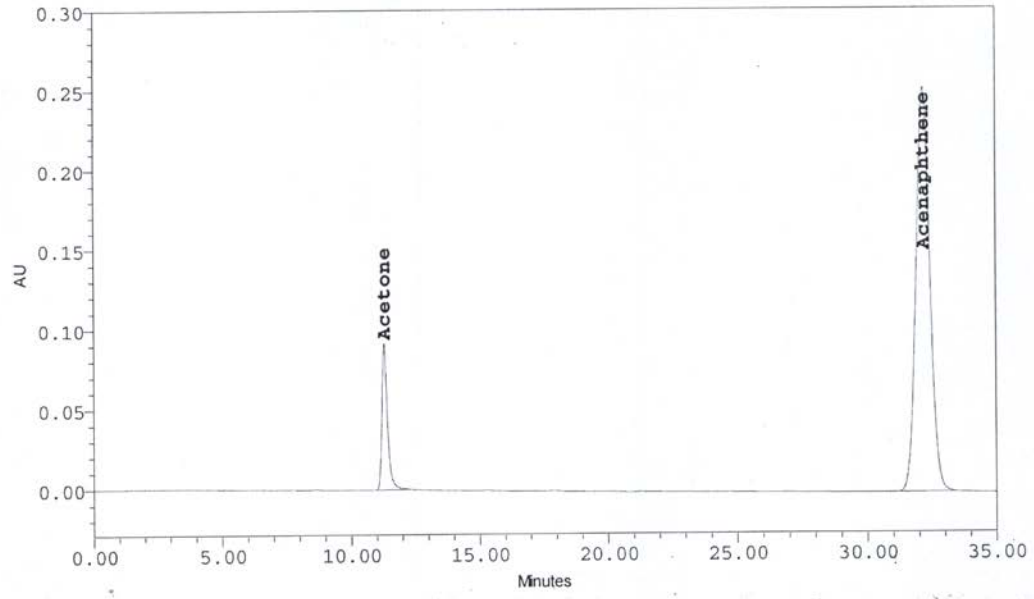
Part Number: 186001166
XTerra® Prep RP18 10.0µm
7.8mm x 300mm Column

Column Serial Number: 127I3222311906 Batch Number: 127I

Test Method

Mobile Phase:	70/30 Acetonitrile/Water
Sample:	Acetone 8 µL/mL
	Acenaphthene 1.2 mg/mL
Flow Rate:	0.8 mL/min.
Detection:	254 nm
Temperature:	Ambient
Injection Volume:	5.0µL

System Name: System 29



Test Results For Acenaphthene

USP Tangent Efficiency: 17361

USP Tailing: 1.03

Pressure (Psig): 198

Retention Time: 32.17

Capacity Factor: 1.85

Accepted By: 346

Date: 13 August 2012 13:45:01 Europe/Dublin

1% Oxygen in Nitrogen Gas

MATHESON TRI•GAS
ask. . .The Gas Professionals™

Certified Mixture Grade

MANUFACTURED AT:
61 Grove Street
Gloucester, MA 01930

SHIPPED BY:
Matheson Tri-Gas Inc.
603 Heron Drive
Bridgeport, NJ 08014
Phone:
Fax:

TO: ROBERTS OXYGEN CO , MD
17011 RAILROAD STREET
GAITHERSBURG, MD 20877

PHONE:
FAX:

TO AVOID BACKFILL, CYLINDER PRESSURE MUST BE GREATER THAN PROCESS PRESSURE

SALES ORDER NUMBER: 368096
P.O. NUMBER: 210825
LOT NUMBER: 107-66-01009

PRODUCT: 2 COMPONENT GAS MIXTURE, CERTIFIED

CYLINDER NUMBER: 42664T
SIZE: 1A
CGA/DISS OUTLET: 580
CONTENT: 200 cu. ft.
PRESSURE: 2000 psia

FILL DATE: May 2, 2006
EXPIRATION DATE: May 2, 2009

COMPONENT	REQUESTED CONCENTRATION	BLEND TOLERANCE (+/-)	CERTIFIED CONCENTRATION	CERTIFICATION ACCURACY
OXYGEN, UHP	1.00 %	10 %	0.998 %	+/- 2%
NITROGEN, UHP	balance		balance	

TRACEABLE TO REFERENCE STANDARD SOURCE/NUMBER:
TRACEABLE TO NIST TRACEABLE WEIGHT CERTIFICATE:

SPECIAL INFORMATION / ADDITIONAL COMMENTS

The product listed above and furnished under the referenced purchase order has been tested and found to contain the component concentration listed above. All values in mole/mole basis gas phase unless otherwise indicated. Matheson Tri-Gas Inc. warrants that the above product(s) conform at the time of shipment to the above description. Matheson Tri-Gas Inc. liability does not exceed the value of the product purchased.

Annie Keane		May 4, 2006
ANALYST	SIGNATURE	DATE SIGNED

Page 1 of 1

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: 8/28/2012

Nitrogen, Ultra High Purity Carrier Grade

01/23/2007 12:53 3019482465 ROBERTS OXYGEN PAGE 02

ROBERTS OXYGEN

ROCKVILLE, MARYLAND 20855
TEL: (301) 948-8100

CERTIFICATE OF CONFORMANCE

THIS DOCUMENT CERTIFIES THAT THE PRODUCT SUPPLIED BY ROBERTS OXYGEN COMPANY, INC. COMPLIES WITH THESE CURRENT PURITY SPECIFICATIONS.

NITROGEN, ULTRA HIGH PURITY CARRIER GRADE

ANALYSIS	GUARANTEE
Nitrogen(N_2)	≥ 99.999%
Oxygen (O_2)	less than 0.0002% (< 2 PPM)
Moisture (H_2O)	less than 0.0002% (< 2 PPM)
Total Hydrocarbons	less than 0.00005% (< .5 PPM)
Hydrogen (H_2)	less then 0.0005% (< 5 PPM)
Dew Point	-71 degrees C @ atmospheric pressure

Specifications listed above meet or exceed military specification MIL-PRF-27401D grade C

Produced by Air Liquefaction Process

12/22/05

Helium

MATHESON TRI•GAS
ask. . .The Gas Professionals™

Certificate of Analysis

Customer Service Center
166 KEYSTONE DRIVE
MONTGOMERYVILLE, PA 18936
P 800-416-250
F 215-619-0458
info@matheson-trigas.com

Manufacturing Location
61 GROVE STREET
GLOUCESTER, MA 01930

Shipping Location
603 HERON DR
BRIDGEPORT, NJ 08014

CYLINDER NUMBER(S)
*050523T

To: ROBERTS OXYGEN CO
17011 RAILROAD ST
GAITHERSBURG, MD 20877

Certificate No: 20005
MTG Customer No: 747635
Sales Order No: 372714
Purchase Order No: 214085

Product: HELIUM
Grade: RESEARCH 99.9999%
MTG Part No: G2137101
Cust Part No: MS121371018

Cylinder Size: 1A
Valve: CGA 580 BR
Contents:
Net: 244 ft³; 6.91 m³
Pressure: 2490 psig @ 70°F; 17269 kPa @ 21°C

	Specification	Certified Concentration	Units
Helium	≥ 99.9999	≥ 99.9999	%
Carbon Dioxide	< 0.1	ND < 0.1	ppm
Carbon Monoxide	< 0.1	ND < 0.05	ppm
Nitrogen	< 0.4	ND < 0.05	ppm
Oxygen	< 0.1	ND < 0.05	ppm
THC as Methane	< 0.1	ND < 0.1	ppm
Water	< 0.2	0.14	ppm

COMMENTS

*** Indicates the actual cylinder(s) analyzed.**

Lot No: 1076201202A3
Fill Date: 05/25/2006
Expiration Date: 05/25/2009

The provision of this certificate is expressly subject to and governed by Matheson Tri-Gas' Terms and Conditions of Sale available on our web site, www.matheson-trigas.com. This document was issued electronically and data validated by electronic signature.

Mike Piotrowicz - Analyst — 05/26/2006 Date

Kimberly Harbeson - QA Approval — 06/15/2006 Date

Page 1 of 1

Iodine

Certificate of Analysis **Page 1 of 1**

Fisher Scientific

Fisher Chemical

1 Reagent Lane
Fairlawn, NJ 7410
201.796.7100 tel
201.796.1329 fax

Certificate of Analysis

Fisher Scientific's Quality System has been found to conform to Quality Management System Standard ISO9001:2000 standard by DNV Certificate number CERT-08052-2003-AQ-HOU-RAB

This is to certify that units of the above mentioned lot number were tested and found to comply with the specifications of the grade listed. Certain data have been supplied by third parties. Fisher Scientific expressly disclaims all warranties, expressed or implied, including the implied warranties of merchantability and fitness for a particular purpose. Certain products (USP/FCC/NF/EP/BP/JP grades) are sold for use in food, drug, or medical device manufacturing. Fisher does not claim regulatory coverage under 21 CFR nor maintain DMF's with the FDA. The following are the actual analytical results obtained:

Catalog Number	I37	**Mfg. Date**	1/17/2006
Lot Number	056529		
Description	IODINE, A.C.S.		

Result name	Units	Specifications	Test Value
APPEARANCE		REPORT	Dark metallic flakes
ASSAY	%	>= 99.8	99.9
CHLORINE & BROMINE	%	<= 0.005	0.003
IDENTIFICATION	PASS/FAIL	= PASS TEST	PASS TEST
NONVOLATILE MATTER	%	<= 0.01	0.010

CERTIFIED BY Edgar E. Hare

Lab Manager Fairlawn

Note: The data listed is valid for all package sizes of this lot of this product, expressed as a extension of this catalog number listed above. If there are any questions with this certificate, please call Chemical Services at (800) 227-6701.

Sterile Water, Injection, USP

APP PHARMACEUTICALS
3159 Staley Road, Grand Island, NY 14072, (716) 773-0800

Certificate of Analysis

Finished Product

Product Name:	Sterile Water for Injection, USP		
Product Code:	918510	**Lot Number:**	405313
Manufacture Date:	03/05/08		
Release Date:	04/15/08	**Expiration Date:**	03/11

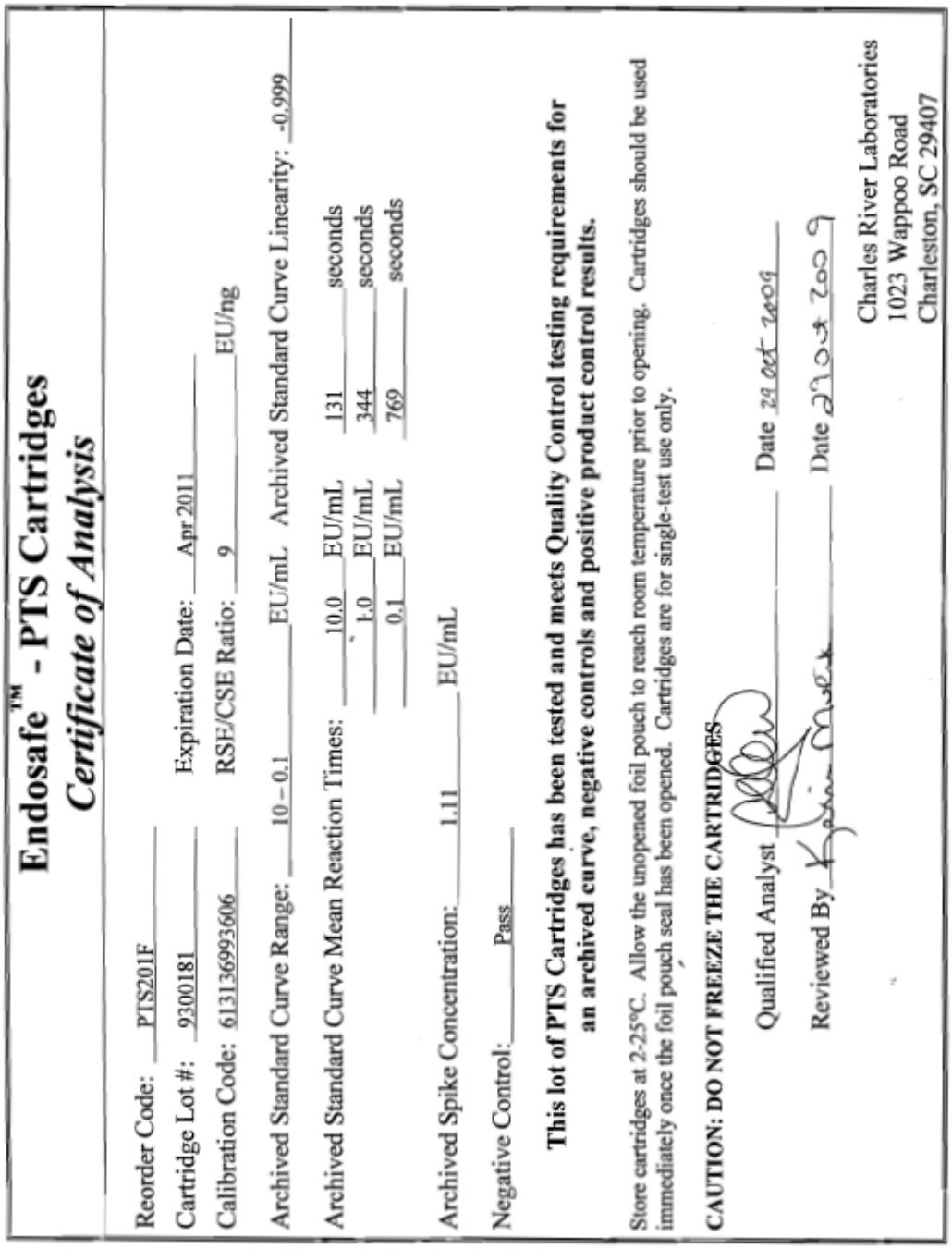
Analysis	Specifications	Results	Conforms
Ammonia	NMT 0.6 ppm	NMT 0.6 ppm	✓
Calcium	No turbidity is produced	Meets Specifications	✓
Carbon Dioxide	The mixture remains clear	Meets Specifications	✓
Chloride	NMT 0.5 ppm	NMT 0.5 ppm	✓
Color of Solution	Compares to Standard	Compares to Standard	✓
Labeling	Label it to indicate that no antimicrobial or other substance has been added, and that it is not suitable for intravascular injection without its first having been made approximately isotonic by the addition of a suitable solute	Meets Specifications	✓
Oxidizable Substances	The pink color does not completely disappear	Meets Specifications	✓
PH	5.0 – 7.0	5.8	✓
Sulfate	No turbidity is produced	Meets Specifications	✓
Visual Inspection 1. Container/Closure 2. Color/Clarity	 1. Container is intact 2. Clear, no particulate matter present	 1. Container is intact 2. Clear, no particulate matter present	 ✓ ✓
Volume in Container 10 mL Label Claim	NLT Label Claim	NLT Label Claim	✓
Other Requirements	Meets Requirements of USP <I>	Meets Requirements of USP <I>	✓
Sterility	Sterile	Sterile	✓
Particulate Matter	1. Does not exceed 6000 particles ≥ 10 microns per container 2. Does not exceed 600 particles ≥ 25 microns per container	1. 41 2. 0	✓ ✓
Bacterial Endotoxins	NMT 0.25 EU/mL	<0.05 EU/mL	✓

This product conforms to the specifications established by
APP Pharmaceuticals

4/15/08
Product Release Management

8/12/08

Endosafe PTS Cartridges



Endosafe™ - PTS Cartridges

Certificate of Analysis

Reorder Code: PTS201F

Cartridge Lot #: 9300181 Expiration Date: Apr 2011

Calibration Code: 613136993606 RSF/CSE Ratio: 9 EU/ng

Archived Standard Curve Range: 10 – 0.1 EU/mL Archived Standard Curve Linearity: 0.999

Archived Standard Curve Mean Reaction Times:			
	10.0 EU/mL	131	seconds
	1.0 EU/mL	344	seconds
	0.1 EU/mL	769	seconds

Archived Spike Concentration: 1.11 EU/mL

Negative Control: Pass

This lot of PTS Cartridges has been tested and meets Quality Control testing requirements for an archived curve, negative controls and positive product control results.

Store cartridges at 2-25°C. Allow the unopened foil pouch to reach room temperature prior to opening. Cartridges should be used immediately once the foil pouch seal has been opened. Cartridges are for single-test use only.

CAUTION: DO NOT FREEZE THE CARTRIDGES

Qualified Analyst _____ Date 29 Oct 2009

Reviewed By _____ Date 29 Oct 2009

Charles River Laboratories
1023 Wappoo Road
Charleston, SC 29407

CA-PTS20F-01

Eppendorf Sterile Pipette Tips

ACILA AG

E-Post: service@acila.com / www.acila.com
St.-Nr. 00722805535/Ust.-Id.: DE111632765

ACILA AG – Opelstrasse 14 – D-64546 Mörfelden-Walldorf

Eppendorf Polymere GmbH
Qualitätssicherung
Sebenter Weg 39
23758 Oldenburg

ZERTIFIKAT Eppendorf-BIOPUR QUALITÄT /
CERTIFICATE of Eppendorf-BIOPUR QUALITY

STERILITÄT / STERILITY

Methode / method	:	Beta-Strahlen gemäß EN ISO 11137 bei einem SAL von 10^{-6} / Beta-radiation according EN ISO 11137 with SAL (Sterility Assurance Level) of 10^{-6}
Prüfverfahren / test method	:	USP, Ph. Eur.

ENDOTOXIN

Prüfverfahren / test method	:	Kinetisch-turbidimetrischer LAL-Test / Kinetic-turbidimetric LAL-Test (FDA Guideline)
Grenzwert / limit	:	< 0,001 EE (EU; I.E.; I.U.) / mL

ATP

Prüfverfahren / test method	:	Bioburden Test / Pre-radiation bioburden Test
Grenzwert / limit	:	< 5,5 X 10^{-10} mg

Die Charge genügt den Anforderungen der USP und Ph. Eur. im Hinblick auf Sterilität. Die Grenzwerte für Endotoxin und ATP werden nicht überschritten.
This product lot meets both USP and Ph. Eur. requirements for sterility. The concentration levels for Endotoxin and ATP are less than the above stated limits.

20.02.08 — Datum / date
P10038.3 — Eingangsnummer / registration number
Dr. Weidner

Die Prüfergebnisse beziehen sich ausschließlich auf die Prüfgegenstände. Das Zertifikat darf auszugsweise nicht ohne schriftliche Genehmigung der ACILA AG vervielfältigt werden.
The test results refer exclusively to the items tested. The certificate must not be copied partially without the approval of ACILA AG.

\\Zeus\Daten\Zertifizierungsamt\Analysenzertifikate\Vorlagen\BIOPUR Zertifikat.doc

Verwaltung & Vertrieb
Headquarter & Sales
Dresdenweg 6
D-64546 Walldorf
Tel.: +49-172-9348819
Fax: +49-6105-277361

Produktion & Labor Mörfelden
(Eingangspr. beginnt mit M)
Opelstrasse 14
D-64546 Mörfelden-Walldorf
Tel.: +49-6105-9301-0
Fax: +49-6105-9301-15

Vorstand: Dr. Peter Weidner
Amtsgericht Darmstadt: HRB 54830
Postbank Dortmund (BLZ 440 100 46), Konto-Nr. 632 402 468
IBAN: DE90 4401 0046 0632 4024 68 / BIC: PBNKDEFF
Frankfurter Volksbank (BLZ 501 900 00), Konto-Nr. 410 154 1652
IBAN: DE15 5019 0000 4101 5416 52 / BIC: FFVBDEFF

Certificate of purity – Biopur® – Quality
Reinheitszertifikat – Biopur® – Qualität

LADR GmbH
Labormedizinisches Versorgungszentrum Bremen
Bereich Lebensmittel- & Umweltanalytik
Leitung: Dr. rer. nat. Uwe Schröder

accredited by
Deutscher Akkreditierungs Rat
DAR
DAC-P-0260-04-00

epT.I.P.S. Singles 2-200 µl
100 Eppendorf Tips individually wrapped Eppendorf Biopur

LOT X131232H

2013 -02

Eppendorf AG
22331 Hamburg · Germany
Tel. +49 1803 666 789
E-Mail: eppendorf@eppendorf.com

Distributed in North America by
Eppendorf North America · USA
Tel. 800 645 3050
E-Mail: info@eppendorf.com

Order no.: 0030 010.035 ***Cat. no.: 022491148***

- **Human DNA / humane DNA**
  Method / Methode: Polymerase Chain Reaction
  Limit / Grenzwert: < 2 pg; less than one human cell / weniger als eine humane Zelle
- **Bacterial DNA / bakterielle DNA**
  Method / Methode: Polymerase Chain Reaction
  Limit / Grenzwert: < 50 fg; less than 10 *E. coli* cells / weniger als 10 *E. coli* Zellen
- **RNase**
  Method / Methode: RNA Digestion / RNA Verdau
  Limit / Grenzwert: not detectable / nicht nachweisbar (LOD / Nachweisgrenze: 1.0 x 10^{-9} Kunitz-units)

Testing of the above described lot showed conformity within the limits of detection. The Lot is released for use.
Die Prüfung des oben ausgewiesenen Lots ergab eine Einhaltung der Grenzwerte. Das Lot wird hiermit freigegeben.

EN Validated 17025

ISO Certified 9002

12.02.2008 — Date / Datum

FPP 0802 1771 — Registration number / Eingangsnummer

Dr. rer. nat. U. Schröder

The test results refer exclusively to the items tested. The certificate must not be copied partially without the approval of LADR GmbH.

Die Prüfergebnisse beziehen sich ausschließlich auf die Prüfgegenstände. Das Zertifikat darf auszugsweise nicht ohne schriftliche Genehmigung der LADR GmbH vervielfältigt werden.

LADR GmbH Bereich Lebensmittel & Umweltanalytik
Bremen | Germany | Phone: +49 421 43 07-500 | Fax: +49 421 4307-199 | E-Mail: info@lanisa.de

Bactec Standard Anaerobic/F Medium

BD

Becton Dickinson Caribe LTD.
PO Box 372860
Cayey PR 00737-2860 PR

Certificate of Analysis

Page: 1 of 2

Product Name	: BACTEC STD ANAER/F 50PK F/G		
Catalog Number	: 442191	**Manufacture Date**	: 2009/12/18
Batch Number	: 9327386		
Expiration Date	: 2010/09/30		

This is to certify that representative samples of
BACTEC STANDARD ANAEROBIC/F MEDIUM
were tested in the Quality Control Laboratory by procedures conventionally utilized for this type of product, including methodology and control ATCC cultures specified in the CLSI standard, Quality Assurance for Commercially Prepared Microbiological Culture Media*, and met the following test parameters:

pH: 7.2 ± 0.2

Autoclaving:
The product was exposed to a moist heat sterilization process (previously validated following an ISO Standard**).

Vacuum draw:
greater than or equal to 5 ml at time of manufacture.

Biological Performance:
Satisfactory growth:

CULTURE	ATCC No.
***Bacteroides fragilis	25285
Bacteroides vulgatus	8482
Clostridium histolyticum	19401
C. perfringens	13124
Escherichia coli	25922
Staphylococcus aureus	25923
***Streptococcus pneumoniae	6305

ATCC is a trademark of the American Type Culture Collection.

* Clinical and Laboratory Standards Institute. 2004. Approved Standard, M22-A3. Quality assurance for commercially prepared microbiological culture media, 3rd ed. CLSI, Wayne, PA.

** ISO 11134, Sterilization of health care products - Requirements for validation and routine control - Industrial moist heat sterilization, 1994.

***CLSI Strain

The Batch Number on this certificate is synonymous with the Lot Number shown on the product label.

Creation Date: 2010/01/29 17:21:29

BD

Becton Dickinson Caribe LTD.
PO Box 372860
Cayey PR 00737-2860 PR

Certificate of Analysis

Page: 2 of 2

Product Name	: BACTEC STD ANAER/F 50PK F/G		
Catalog Number	: 442191	Manufacture Date	: 2009/12/18
Batch Number	: 9327386		
Expiration Date	: 2010/09/30		

BD Diagnostic Systems (BDDS) is an ISO 13485:2003 and ISO 9001:2000 Registered facility. BDDS products are manufactured in facilities registered with the United States Food and Drug Administration (FDA), and are regulated by the FDA's Quality System Regulations (QSRs). This product met BDDS stringent quality standards at time of batch/lot release. Any test results reported on this certificate were obtained at time of release.

John Gerlich
Vice President,
Quality Management and
Regulatory Compliance
Signature Date: 2010/01/29

Creation Date: 2010/01/29 17:21:29

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: 8/28/2012

Bactec Standard/10 Aerobic/F Medium

BD

Becton Dickinson Caribe LTD.
PO Box 372860
Cayey PR 00737-2860 PR

Certificate of Analysis

Page: 1 of 2

Product Name : MEDIUM BACTEC STD/10 AEROBIC
Catalog Number : 442260 **Manufacture Date** : 2009/12/18
Batch Number : 9328160
Expiration Date : 2010/09/30

This is to certify that representative samples of
BACTEC STANDARD/10 AEROBIC/F MEDIUM
were tested in the Quality Control Laboratory by procedures conventionally utilized for this type of product, including methodology and control ATCC cultures specified in the CLSI standard, Q uality Assurance for Commercially Prepared Microbiological Culture Media*, and met the following test parameters:

pH: 7.2 ± 0.2

Autoclaving:
The product was exposed to a moist heat sterilization process (previously validated following an ISO Standard**).

Vacuum draw:
greater than or equal to 10 ml at time of manufacture.

Biological Performance:
Satisfactory growth:

CULTURE	ATCC No.
Alcaligenes faecalis	8750
Candida albicans	18804
Escherichia coli	25922
Haemophilus influenzae	19418
Neisseria meningitidis	13090
***Pseudomonas aeruginosa	27853
Staphylococcus aureus	25923
***Streptococcus pneumoniae	6305
S. pyogenes Group A	19615

ATCC is a trademark of the American Type Culture Collection.

* Clinical and Laboratory Standards Institute. 2004. Approved Standard, M22-A3. Quality assurance for commercially prepared microbiological culture media, 3rd ed. CLSI, Wayne, PA.

** ISO 11134, Sterilization of health care products - Requirements for validation and routine control - Industrial moist heat sterilization, 1994.

***CLSI Strain

Creation Date: 2010/01/29 16:26:44

BD

Becton Dickinson Caribe LTD.
PO Box 372860
Cayey PR 00737-2860 PR

Certificate of Analysis

Page: 2 of 2

Product Name	: MEDIUM BACTEC STD/10 AEROBIC		
Catalog Number	: 442260	Manufacture Date	: 2009/12/18
Batch Number	: 9328160		
Expiration Date	: 2010/09/30		

The Batch Number on this certificate is synonymous with the Lot Number shown on the product label.

BD Diagnostic Systems (BDDS) is an ISO 13485:2003 and ISO 9001:2000 Registered facility. BDDS products are manufactured in facilities registered with the United States Food and Drug Administration (FDA), and are regulated by the FDA's Quality System Regulations (QSRs). This product met BDDS stringent quality standards at time of batch/lot release. Any test results reported on this certificate were obtained at time of release.

John Gerlich
Vice President,
Quality Management and
Regulatory Compliance
Signature Date: 2010/01/29

Creation Date: 2010/01/29 16:26:44

[^{11}C]ER176 Injection: Validation Record

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: 9/12/12

Three batches of *[^{11}C]ER176 Injection* were produced and fully analyzed for the purpose of validation of the process. The data are summarized below; refer to the three batch records attached for further detail.

pH	**Batch Number**	**Result**
	ER176-12090501	4.5
Acceptance Criteria: pH 4.0 to 7.5	ER176-12091101	5.0
	ER176-12091102	5.0

Membrane filter integrity	**Batch Number**	**Result**
	ER176-12090501	No bubbles observed at 45 psi
Acceptance Criteria: No bubbles observed at 45 psi	ER176-12091101	No bubbles observed at 45 psi
	ER176-12091102	No bubbles observed at 45 psi

Appearance test	**Batch Number**	**Result**
	ER176-12090501	Clear, colorless, particulate free
Acceptance Criteria: Clear, colorless, particulate free	ER176-12091101	Clear, colorless, particulate free
	ER176-12091102	Clear, colorless, particulate free

Chemical purity	**Batch Number**	**Result**
	ER176-12090501	0.0696 µg
Acceptance Criteria: Amount of impurity not to exceed 1.0 µg for injected volume.	ER176-12091101	0.0116 µg
	ER176-12091102	0.00820 µg

Cold Carrier Limit	**Batch Number**	**Result**
	ER176-12090501	10.0 µg
Acceptance Criteria: Amount of ER176 carrier not to exceed 10 µg for injected volume.	ER176-12091101	6.08 µg
	ER176-12091102	3.98 µg

Radiochemical purity	**Batch Number**	**Result**
	ER176-12090501	100%
Acceptance Criteria: Not less than 95%	ER176-12091101	100%
	ER176-12091102	100%

Radiochemical identity	**Batch Number**	**Result**
	ER176-12090501	Δ t_R = 0.208 min
Acceptance Criteria: t_R within 1 min of standard (corrected for UV to γ detector delay)	ER176-12091101	Δ t_R = 0.208 min
	ER176-12091102	Δ t_R = 0.192 min

Specific radioactivity	**Batch Number**	**Result**
	ER176-12090501	2965 mCi/µmol
Acceptance Criteria: Not less than 500 mCi/µmol at EOS	ER176-12091101	5444 mCi/µmol
	ER176-12091102	5928 mCi/µmol
Radionuclidic Identity	**Batch Number**	**Result**

[^{11}C]ER176 Injection: Validation Record

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: 9/12/12

	ER176-12090501	$t_{1/2}$ = 20.3
Acceptance Criteria: Experimentally determined half life 20.4 ± 2.0 minutes	ER176-12091101	$t_{1/2}$ = 20.7
	ER176-12091102	$t_{1/2}$ = 19.9

Residual Solvent	**Batch Number**	**Result**
	ER176-12090501	0.102 mg acetonitrile
		5.88 x 10^4 ng/µL ethanol
Acceptance Criteria: ≤ 4.1 mg acetonitrile in the injectable dose ≤1 x 10^5 ng/µL ethanol	ER176-12091101	0.224 mg acetonitrile
		5.73x 10^4 ng/µL ethanol
	ER176-12091102	0.143 mg acetonitrile
		5.04 x 10^4 ng/µL ethanol

Bacterial endotoxins	**Batch Number**	**Result**
	ER176-12090501	< 9.2 EU
Acceptance Criteria: No more than 175 EU in the injectable dose	ER176-12091101	< 19.0 EU
	ER176-12091102	< 16.2 EU

Sterility	**Batch Number**	**Result**
	ER176-12090501	No growth observed
Acceptance Criteria: No aerobic or anaerobic growth observed	ER176-12091101	No growth observed
	ER176-12091102	No growth observed

Stability	**Batch Number**	**Result**
	ER176-12090501	100 %
		Clear, colorless, particulate free
Acceptance Criteria: Product meets radiochemical purity specifications (≥ 95%) and is clear, colorless, particulate free after one hour.	ER176-12091101	100 %
		Clear, colorless, particulate free
	ER176-12091102	100 %
		Clear, colorless, particulate free

Yield	**Batch Number**	**Result**
	ER176-12090501	134.0 mCi
Acceptance Criteria: Greater than 20 mCi at EOS	ER176-12091101	80.5 mCi
	ER176-12091102	58.3 mCi

Radiopharmacy Dose Sheet for [^{11}C]ER176

Date (mm/dd/yy):	09/05/12	
Batch # (ER176-yymmddxx):	ER176-12090501	
Chemist:	YZ/CM	301-451-3928 (lab)
Radioconcentration (mCi/mL) =	15.37	9:46:08 AM
Specific Radioactivity (mCi/μmol) =	2965.0	9:46:08 AM
ER176 Carrier Concentration (μg/mL) =	2.180	
Total Volume of Formulated [^{11}C]ER176 for Injection (mL) =	8.5	
Maximum Allowable Injection Volume (mL) =	4.6	

Enter slope of calibration curve, M (X= μmol, Y= UV peak area):	8.73106E+08	90% Expected = 453023
Date of calibration curve preparation:	8/21/2012	Expected Area = 503359
		110% Expected = 553695
Enter radioactivity in whole dose vial (mCi):	134	
Enter radioactivity in 100 μL aliquot (mCi):	1.54	**Enter End of Synthesis (EOS) Time**
Enter background radioactivity before injection (mCi):	0.003	↓ ↓
Net activity in 100 μL aliquot (mCi) =	1.537	9:46:08 AM
Enter UV peak area of ER176 carrier peak:	452598	
Enter sum of areas of all impurity peaks:	14444	
Percent chemical purity of ER176 =	96.9	
Molecular weight of ER176 =	420.52	
μmol of ER176 in 100 μL aliquot	5.184E-04	
Mass of ER176 in 100 μL aliquot (μg) =	0.21799	
Mass of ER176 equivalent impurity in 100 μL aliquot (μg) =	0.00696	
Maximum allowable injected carrier mass (μg):	10.0	
Maximum allowable injected impurity mass (μg):	1.0	
Maximum allowable injection volume based on carrier mass (mL) =	4.6	Maximum injection volume is the lesser of the two values when calculated from maximum allowable carrier or maximum allowable impurity
Maximum allowable injection volume based on impurity mass (mL) =	14.4	
Second measurement of whole dose vial for half-life calculation (mCi)	113.0	
Time between measurements (min)	5	
Calculated half-life (min)	20.3	

[^{11}C]*ER176 Injection*: Master Batch Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Approved by: V. W. Pike Initials: VWP Date: 08/28/12

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

Batch # ER176-/209050/ Date: 9/5/12

Reagents/solvents/supplies	Lot/Exp	Purpose	Amount used	Date of Preparation/ Date Opened
Dimethyl sulfoxide, anhydrous	SHBB2380V	Solvent	0.4 mL	8/21/12
Potassium hydroxide, 85%	MKBH1644V	Reagent Base	1.05 mg	8/6/12
N-desmethyl-ER176	ER169_040711	Precursor	0.61 mg	4/7/11
Ethyl alcohol, USP 200 proof	053213	Cleaning	As required	8/31/12
Sterile Saline for Injection, USP	6003786 / 4/2014	Formulation	10.0 mL	Single Use
Ethanol, dehydrated	1346 / 6/2013	Formulation	0.5 ml	Single Use
Sterile vial 10 mL	11-115-EV / 11/1/2014	Dose vial	1	Single Use
Sterile Millex-GV filter (vent filter, 0.22 µm, 4 mm diameter)	R1SA53440 / 7/2014	Formulation	1	Single Use
Sterile Millex-MP filter (sterilization filter, 0.22 µm, 25 mm diameter)	R1KA64800 / 9/2014	Formulation	1	Single Use
Sterile needle (21 gauge; 2") for sterile filtration, 2 each and [^{11}C]MeI transfer, 2 each	1272662	Formulation	4	Single Use
Sterile needle (20 gauge; 1.5 inches long) for sterile vent	0244285	Formulation	1	Single Use
Water, HPLC grade	52006 / 1/31/13	HPLC mobile phase	As required	9/3/12
Acetonitrile, HPLC grade	DG292	HPLC mobile phase	As required	8/31/12
Methanol, HPLC grade	096964	HPLC mobile phase	As required	9/3/12
Ammonium hydroxide, 1N	2202548 / 02/13	Mobile phase modifier	1 mL / 1 L water	6/1/12
HPLC column (analytical, Phenomenex, C-18 Luna; 10 µm; 4.6 mm × 250 mm)	600405-6	Analytical HPLC	1	8/21/12
HPLC column (semi-prep, Waters, RP-18 XTerra; 10 µm; 7.8 mm × 300 mm)	127I32223 11906	Prep HPLC	1	9/3/12
1 mM ammonium hydroxide (aq)	09-03-20121 9/17/12	Prep HPLC mobile phase	1L nominal	9/3/12
MeOH:water, 74:26	09-03-20121 9/17/12	Analytical HPLC mobile phase	1L nominal	9/3/12

[^{11}C]ER176 Injection: Master Batch Record

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Bethesda, MD 20892

Date of review: 8/28/2012

Key operation	Initials	Comment/SOP #
Check all gas valves are open and that pressure on regulators are 60, 12 and 22 p.s.i. for nitrogen, helium and hydrogen, respectively	cm	
6-way valve in NEMA box is set to hot-cell 3 and 3-way valve is on "cryo" position	cm	
Check gas collection valve is on "fill" position	cm	
Run prep sequence on GE Microlab Mel box	cm	
Check flow in RMA, RMB, RMC	cm	
Check the helium flow rate. Typical flow is 15 to 25 mL/min	cm	18.6 mL/min
Check integrity of GE Mel box by running leak check 1	cm	2.0 min
Install clean transfer and vent needles at the Trapping station.	Yz	
Verify the ionization chamber with ^{57}Co and ^{137}Cs standards	cm	
Verify balance using a 10-g NIST traceable weight (acceptable range 9.9−10.1 g)	cm	Weight: 10.0 g
Record weight of sterile empty vial on QC Form.	Yz	
Remove slider and empty reaction oven	Yz	
Open Synthia AutoRad Software	Yz	
Load Recipe **ER176IND**	Yz	
Enter Reaction Temperature (80 °C) and Time (300 s) when prompted	Yz	
Ensure preparative column switching valve is set to ER176 column and switch is set to "Prep" and install preparative mobile phase	cm	
Equilibrate the preparative column with 37/63 acetonitrile/1 mM ammonium hydroxide at 8.0 mL/min. Check for leaks. Pressure should be about 3700 p.s.i.	cm	
Ensure analytical column switching valve is set to ER176 column and switch is set to "Analytical" and install analytical mobile phase	cm	
Equilibrate the analytical column with 74/26 MeOH/H_2O at 2.5 mL/min. Check for leaks. Pressure should be about 2400 p.s.i.	cm	
Inject ER176 standard and clean analytical injection port.	Yz	
Test 32Karat Beckman HPLC data acquisition interface box, UV, and PIN diode detector by initiating data collection	cm/Yz	
Connect and wash the product collection line by open the collection valve and flush the Prep mobile phase through the line for at least three minutes.	cm	
Clean all transfer tubing (20-mL column, HPLC fraction collection line, saline inlet line) with USP ethanol and flush dry.	cm	
Verify vacuum integrity. Turn on pump; gauge should read approximately 28 inches of mercury (67mBar)	cm	
Connect a 10-mL syringe containing 5% ethanol in saline to end of addition line.	Yz	
Fill heating bath with water and set to 80 °C	cm	

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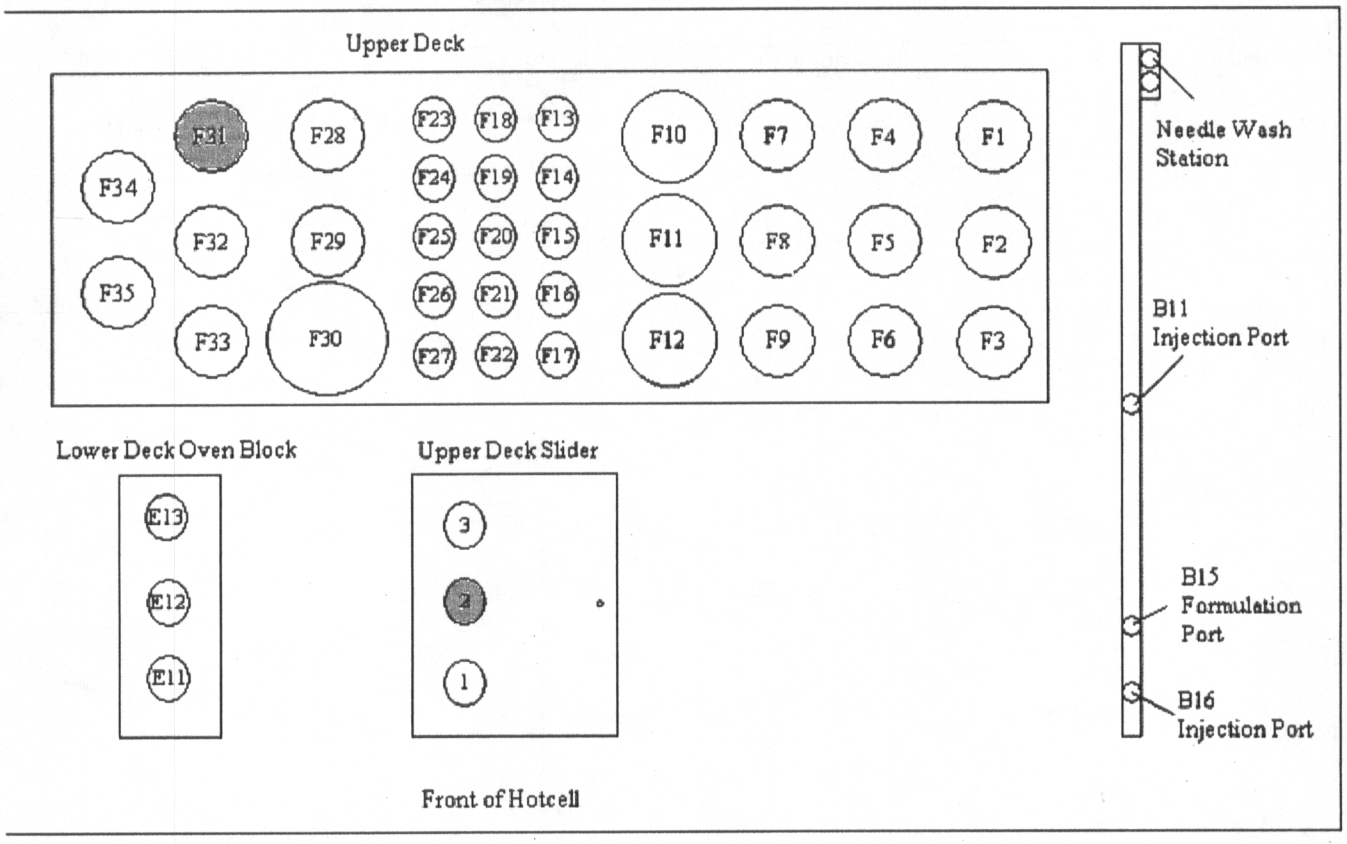
Date of review: 8/28/2012

Check that the dry ice traps are full	cm	
Record weight of sterile empty vial; prepare and install sterile dose vial unit.	YZ / cm	
Check that all waste reservoirs are sufficiently empty	YZ	
Prepare a vial for residual solvent analysis and place in the formulation hot cell	YZ	
Prepare the endotoxin unit for operation	cm	
Prepare solution of ER176 precursor (0.6 ± 0.06 mg) / KOH (1.2 ± 0.2 mg) in DMSO (0.4 mL), crimp seal and place in Slider position 2 underneath vent needle when prompted.	YZ	

Figure 1: Diagram of Synthia Deck Layout



Position	Tube Type	Solvent / Material
F31	10 mL round bottom	70:30 Absolute Ethanol: HPLC grade Water
Slider Position 2	Reaction Vial	Precursor, KOH, DMSO

PET Radiopharmaceutical Sciences Section,
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Date of review: 8/28/2012

Summary:

Cyclotron, run #	GE1, 9847
End of bombardment	9:00
Beam current	45 μA
Bombardment time	40 min
Final formulated product in dose calibrator	134.0 mCi at 9:45:18

Chemist:	Signature:	Date
Cheryl Morse	Cheryl Morse	9/5/2012

[^{11}C]ER176 Injection: Quality Control Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Approved by: V W Pike Initials: VWP Date: 08/28/12
Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Batch # ER176-12090501 Date: 9/5/2012

Quality Control Instrument and Materials Verification			
Verification of Ionization Chamber	**Test Data**	**Acceptance Criteria**	**Acceptance Criteria Met**
^{137}Cs source reference ID: 970-31-4 204 µCi on 3/1/2003 Expected radioactivity 163.9 µCi Acceptable Range: 155.7 µCi to 172.1 µCi	^{137}Cs measured: 157.2 µCi	Does measured radioactivity fall within acceptable range (± 5%) for both ^{137}Cs and ^{57}Co?	(Y) / N
^{57}Co source reference ID: 1393-22-20 5516 µCi on 12/01/2009 Expected radioactivity 420 µCi Acceptable Range: 400 µCi to 441 µCi	^{57}Co measured: 403 µCi		
Verification of Analytical HPLC system			
Calibration curve date: 8/21/12 Slope (area/µmol): 8.731 x10^8 Mass injected: 204 ng Expected peak area: 503359 Acceptable range: 453023 to 553695	Measured peak area: 484506 t_R: 4.842 min	Peak area within ± 10% of expected peak area?	(Y) / N

Post-Production Measurements and Release Tests	
Volume	**Data**
Subtract the weight of the empty dose vial from the full dose vial. Assume the density of the final product is approximately 1 g/mL to determine volume. Weight of full vial: 29.8 g; Weight of empty vial: 21.3 g	Volume of [^{11}C]ER176 Injection: 8.5 mL
Yield	
Measure the activity in the dose vial after removal of the QC sample.	Measured activity: 134.0 mCi at 9:45:18 (EOS)

Test	Test Data	Acceptance Criteria	Criteria Met
pH			
Dispense one to two drops of [^{11}C]ER176 Injection on pH paper.	Measured pH: 4.5	pH measured between 4.0 to 7.5?	(Y) / N
Membrane Filter Integrity			
Attach the syringe filter and needle used for sterile filtration to the compressed air source. Submerge needle tip in water. Pressurize to 45 psi.	Observation: Bubbles / (No Bubbles)	No bubbles observed from the submerged needle tip at 45 psi?	(Y) / N

[^{11}C]ER176 Injection: Quality Control Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
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Bethesda, MD 20892

Date of review: 8/28/2012

Appearance			
Visually inspect the *[^{11}C]ER176 Injection* in the dose vial.	Evaluate appearance	Colorless solution free of particulates?	(Y) / N
Radionuclidic Identity			
Determine the half life experimentally from two time points separated by at least three minutes.	t_1 134.0 mCi at 9:45:18 t_2 113.0 mCi at 9:50:18 Calc. half life: 20.3 min	Experimental half life 20.4 ± 2 min?	(Y) / N
Residual Solvent			
Determine the residual ethanol and acetonitrile in dose by gas chromatography	Ethanol: 5.878×10^4 ng/µL Acetonitrile: 22.26 ng/µL Max. injectable vol: 4.6 mL Acetonitrile in inj. vol: 1.024×10^5 ng	Ethanol ≤ 1×10^5 ng/µL? Acetonitrile ≤ 4.1×10^6 ng?	(Y) / N (Y) / N
Tests Based on HPLC and LC/MS Analysis[1]			
Measure the radioactivity of a 100 µL aliquot of *[^{11}C]ER176 Injection:* 1540 µCi @ 9:46:08 Measure background radiation before injection: 3 µCi			
Radiochemical Identity			
Compare the retention time of *[^{11}C]ER176 Injection* to the ER176 standard retention time.	Standard t_R: 4.842 min Product t_R: 5.050 min	Difference is less than 1.0 min?	(Y) / N
Radiochemical Purity			
Integrate the peaks in the HPLC Bioscan trace. Determine the percent area represented by the product peak.	% Area: 100 %	Greater than 95%?	(Y) / N
Chemical Purity			
Calculate the concentration of carrier and ER176 equivalent impurity in the *[^{11}C]ER176 Injection* from the peak area at 235 nm.	Carrier: 2.18 µg/mL Impurity: 0.0696 µg/mL	Maximum volume contains no more than 10 µg of carrier or 1.0 µg ER176 equivalent impurity.	(Y) / N
Calculate the maximum injection volume as the lesser volume by maximum allowable carrier or maximum allowable equivalent impurity.	Max volume by carrier: 4.6 mL Max volume by impurity: 14.4 mL		
Specific Radioactivity			
Calculate the specific activity of *[^{11}C]ER176 Injection* in units of mCi/µmol	Calculated Specific Activity 2965.0 mCi/µmol	Specific activity ≥ 500 mCi/µmol?	(Y) / N

[1]A calculations worksheet is typically used to perform the calculations required. A copy of the worksheet may be found in Appendix B.

[^{11}C]*ER176 Injection*: Quality Control Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Bacterial Endotoxin Test (Refer to SOP #QA302)

Reagents/Supplies	Lot # / Expiration Date
EndoSafe Unit ID	1247
Test Cartridge Lot Number	2153165 / 6/2014
Sterile Water Lot Number	6002029 / 5/2014
Pipette Tip Lot	A1433265 / 4/2016

	Result	Criteria	Acceptance Criteria Met?
Sample	<2.00 EU/mL Max injectable vol: ~~8.5~~ 4.6 mL EU in injectable vol.: ~~9.2×10~~	≤ 175 EU in total injectable volume YZ 9/15/12	(Y) / N
Sample %CV	~~0.657~~ 0.0%	≤ 25%	(Y) / N
Spike	0.657 YZ 9/15/12	Refer to certificate of analysis of each cartridge lot for theoretical value	NA
Spike %CV	2.9%	≤ 25%	(Y) / N
Recovery	86%	50 – 200%	(Y) / N

All Quality Control specifications met?	(Y) / N
Chemist: Yi Zhang Signature:	Date: 9/5/12

[^{11}C]ER176 Injection: Post-Release Test Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Approved by: V. W. Pike Initials: VWP Date: 08/28/12
Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Batch # ER176-12090501 Date: 9/5/2012

1. Completed vial label
Attach completed vial label to this form.

[^{11}C]ER176 Injection
Sterile, apyrogenic saline solution for intravenous administration.
Caution: New drug limited by Federal law to investigational use only
NIMH MIB Store at room temperature
Expires 1 h after calibration Half-life of ^{11}C is 20.4 min
Concentration: 15.37 mCi/mL Time: 9:46:08
Activity: 134 mCi Volume: 8.5 mL
Date: 9/5/12 Lot #: ER176-12090501

2. Sterility (Reference SOP# QA302)

Procedure:

After radioactivity has decayed, place the formulated dose in the laminar flow cabinet with one Anaerobic (yellow-cap) and one Aerobic (blue-cap) Bactec test vial. Using aseptic technique, transfer approximately 100 µL formulated dose to each Bactec vial. Record the lot numbers and expiration dates on the sample submission form and take samples and form to the NIH Clinical Center Microbiology Lab.

Results:

Sample form should be returned by Microbiology Lab with test results within 2-4 weeks from date of submission. File results with this form.

Is the *[^{11}C]ER176 Injection* negative for aerobic and anaerobic growth? **Circle one:** (Yes) **or No**

Chemist:	Signature:	Date:
Yi Zhang		9/19/12

REQUEST FOR STERILITY TEST

MIB
Molecular Imaging Branch
-NIMH-

Product: C-11 ER176

Date Submitted for Sterility Testing : 9/06/2012

Lot # : C-11 ER176 -12090501

PHYSICAL AND CHEMICAL CHARACTERISTICS: **NON-RADIOACTIVE MATERIAL**

PRESERVATIVE: **NONE**

REMARKS:

Anaerobic (Yellow) Lot# 2115242, exp. 2013-01-31

Aerobic (Blue) Lot# 2093098, exp. 2013-01-31

RESULTS: NO GROWTH AFTER 7 DAYS

SIGNATURE: [signature] DATE: 9/14/12

THIS PRODUCT WAS SUBMITTED FOR STERILITY TESTING BY:

Yi Zhang **301-451 3928**
Bldg. 10, Room B3C355, MSC 1003

IF THERE ARE ANY QUESTIONS OR PROBLEMS CONCERNING THIS PRODUCT, PLEASE CONTACT THE ABOVE INDIVIDUALS.

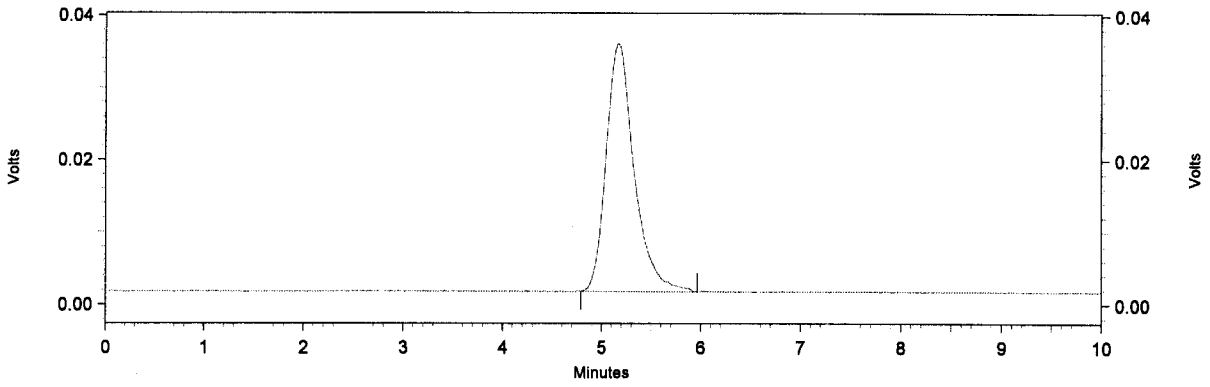
ANALYTICAL HPLC of ER176

Area % Report

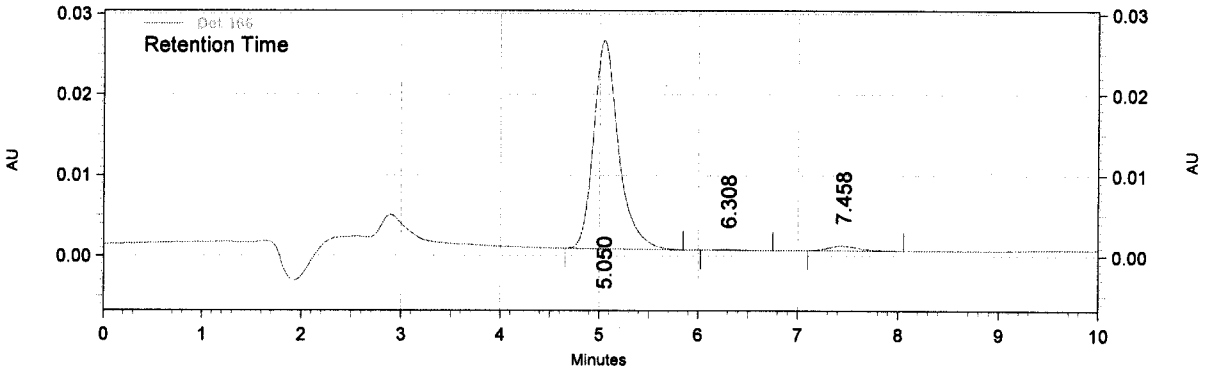
Data File: D:\32Karat\Projects\ER176\Data for IND_Human\Human\ER176-12090501 validation qc
Method: D:\32Karat\Projects\ER176\Method for IND\ER176 Analytical.met
Acquired: 9/5/2012 9:50:52 AM

HPLC: 74/26 MeOH / water, Luna C18, 4.6 x 250, 10micron
UV = 235 nm, flow rate = 2.5ml/min, detector PMT at 20M, Pressure 2.40kpsi

Bioscan



UV at 235 nm



bioscan Results

Time	Area	Area %	Height	Height %
5.166	675178	100.00	34261	100.00

Det 166 Results

Time	Area	Area %	Height	Height %
5.050	452598	96.91	25832	97.32
6.308	2148	0.46	116	0.44
7.458	12296	2.63	596	2.25

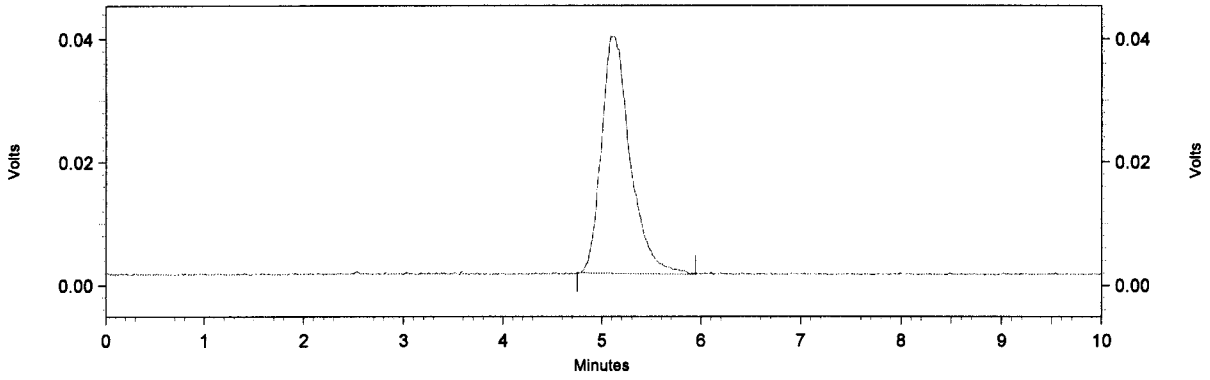
ANALYTICAL HPLC of ER176

Area % Report

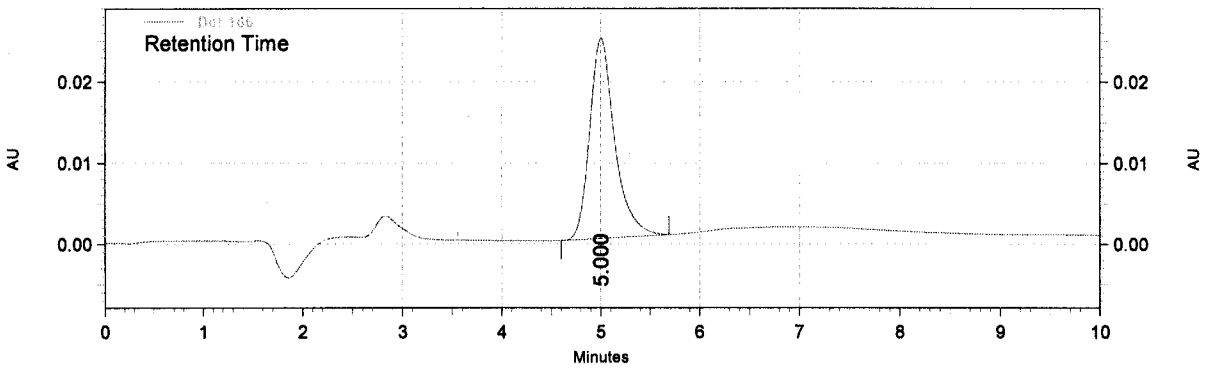
Data File: D:\32Karat\Projects\ER176\Data for IND_Human\Human\ER176-12090501 validation 1hr stability
Method: D:\32Karat\Projects\ER176\Method for IND\ER176 Analytical.met
Acquired: 9/5/2012 10:53:33 AM

HPLC: 74/26 MeOH / water, Luna C18, 4.6 x 250, 10micron
UV = 235 nm, flow rate = 2.5ml/min, detector PMT at 2M, Pressure 2.40kpsi

Bioscan



UV at 235 nm



bioscan Results

Time	Area	Area %	Height	Height %
5.091	775816	100.00	38488	100.00

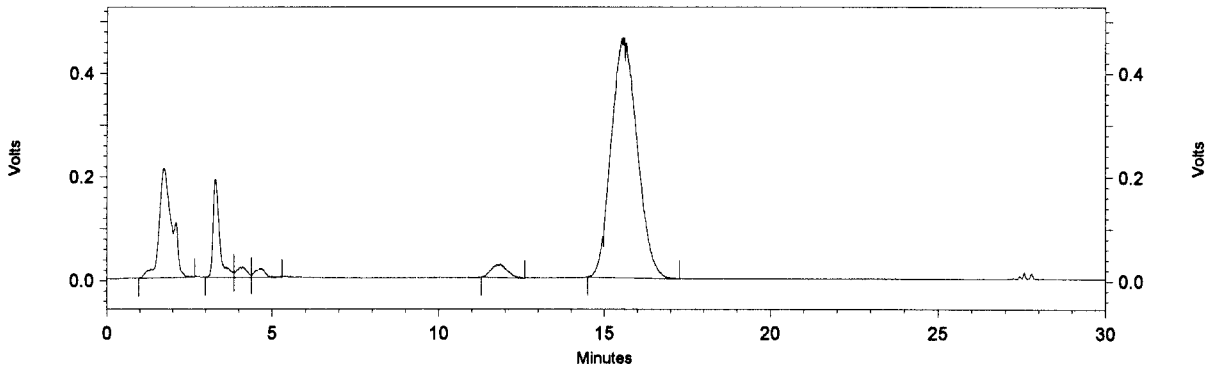
Det 166 Results

Time	Area	Area %	Height	Height %
5.000	433966	100.00	24643	100.00

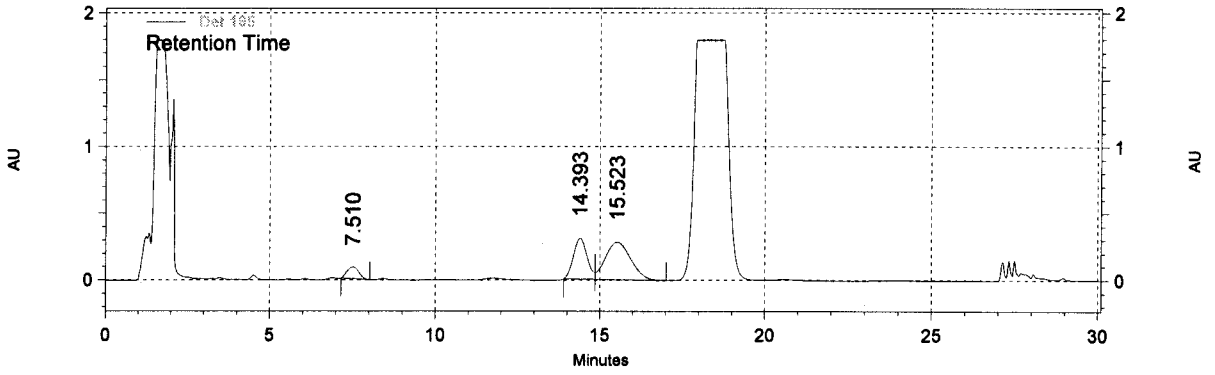
C-11 ER176 via HC#4

Data File: D:\32Karat\Projects\dLop\Data\Human\2012\ER176-12090501 validation Prep.dat
Method: D:\32Karat\Projects\ER176\Method\ER176 IND\ER176 Prep.met
Acquired: 9/5/2012 9:20:00 AM
HPLC: Isocratic B/A = 37/63 MeCN/ 1 mM NH4OH; Flow rate = 8.0 ml/min; UV at 235 nm
Waters Xterra, RP18, 10 micron 7.8 mm x300 mm, Pressure 3.7kpsi.

Bioscan



UV 254



Bioscan Results

Time	Area	Area %	Height	Height %
1.723	5748585	16.38	211843	22.84
3.306	2537875	7.23	189225	20.40
4.053	417566	1.19	19084	2.06
4.665	367712	1.05	17646	1.90
11.753	868658	2.47	25625	2.76
15.556	25160552	71.68	464149	50.04

Totals				
	35100948	100.00	927572	100.00

Det 166 Results

Time	Area	Area %	Height	Height %
7.510	2158910	8.25	88964	13.10
14.393	8821544	33.71	307396	45.25
15.523	15190094	58.04	282996	41.66

Totals				
	26170548	100.00	679356	100.00

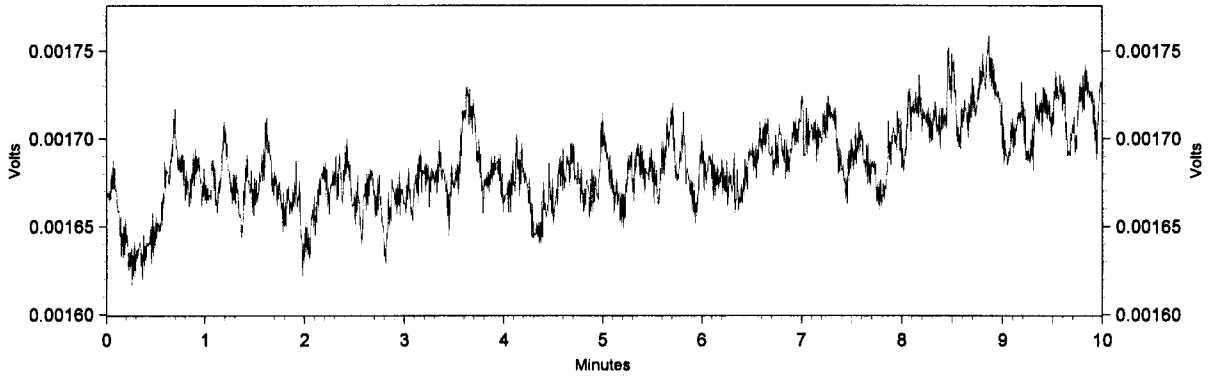
ANALYTICAL HPLC of ER176

Area % Report

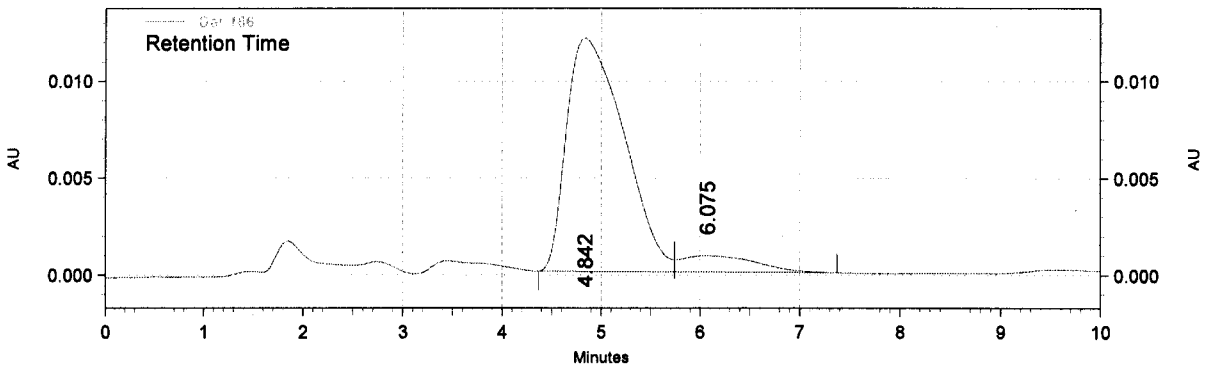
Data File: D:\32Karat\Projects\ER176\Data for IND_Human\Human\ER176-12090501 validation std
Method: D:\32Karat\Projects\ER176\Method for IND\ER176 Analytical.met
Acquired: 9/5/2012 8:10:13 AM

HPLC: 74/26 MeOH / water, Luna C18, 4.6 x 250, 10micron
UV = 235 nm, flow rate = 2.5ml/min, detector PMT at 20M, Pressure 2.40kpsi

Bioscan



UV at 235 nm



bioscan Results

Time	Area	Area %	Height	Height %

Det 166 Results

Time	Area	Area %	Height	Height %
4.842	484506	91.72	12033	93.47
6.075	43740	8.28	841	6.53

ANALYTICAL HPLC of ER176

Area % Report

Data File: D:\32Karat\Projects\ER176\Data for IND_Human\Human\ER176-12090501 validation formulation vehicle
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Acquired: 9/5/2012 8:32:04 AM

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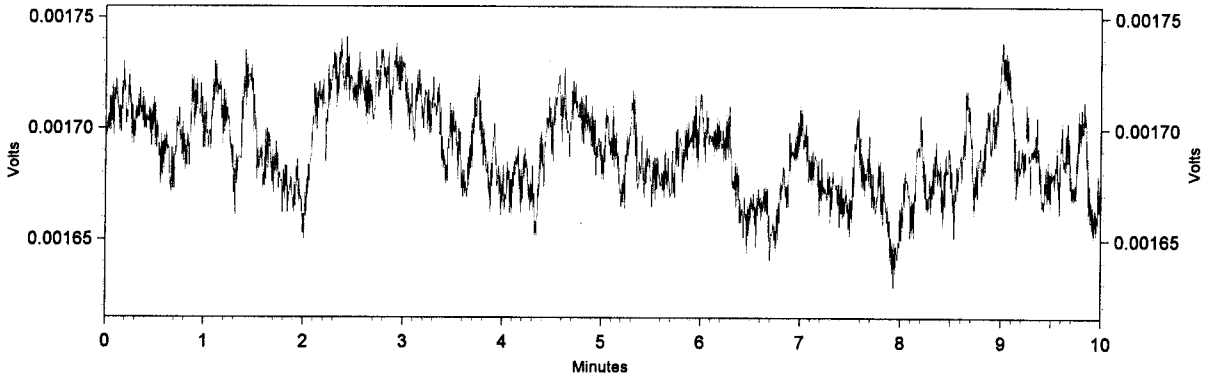
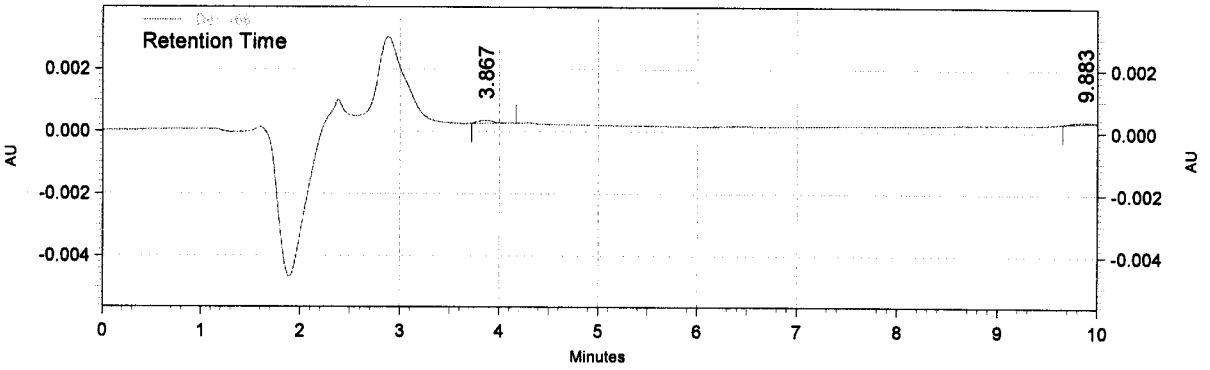
UV = 235 nm, flow rate = 2.5ml/min, detector PMT at 20M, Pressure 2.40kpsi

Bioscan



UV at 235 nm



bioscan Results

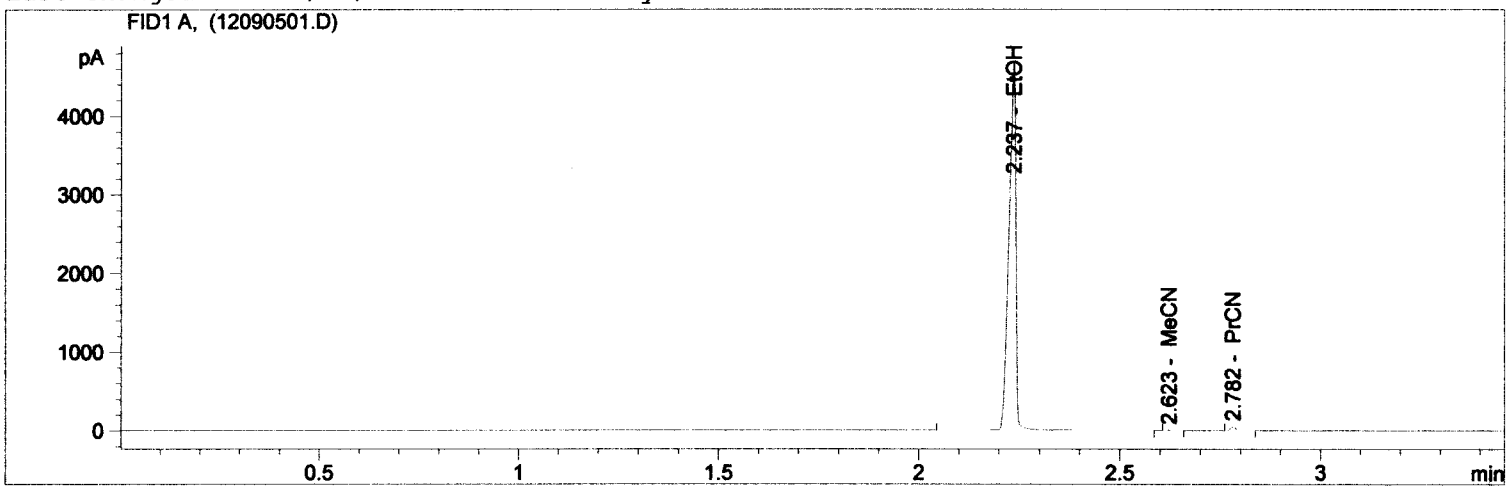
Time	Area	Area %	Height	Height %

Det 166 Results

Time	Area	Area %	Height	Height %
3.867	1095	64.68	98	68.53
9.883	598	35.32	45	31.47

Data File C:\CHEM32\1\DATA\ER176\STD 2012-09-05 10-03-19\12090501.D
Sample Name: ER176-12090501

```
================================================================================
Acq. Operator   : YZ                                  Seq. Line :   1
Acq. Instrument : Instrument 1                         Location : Vial 1
Injection Date  : 05-Sep-12, 10:04:37                      Inj :   1
                                                     Inj Volume : 1 µl
Sequence File   : C:\Chem32\1\DATA\ER176\STD 2012-09-05 10-03-19\STD.S
Method          : C:\CHEM32\1\DATA\ER176\STD 2012-09-05 10-03-19\ISPRCN.M (Sequence Method)
Last changed    : 5/26/2010 8:58:33 AM by clm
```




```
================================================================================
                        Internal Standard Report
================================================================================

Sorted By             :      Signal
Calib. Data Modified  :      12/13/2007 11:12:57 AM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :    386.00000  []   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
Sample ISTD Information:
ISTD  ISTD Amount   Name
  #
----|------------|-------------------------
  1    386.00000   PrCN


Signal 1: FID1 A,

RetTime  Type  ISTD    Area      Amt/Area    Amount   Grp   Name
 [min]         used  [pA*s]      ratio
-------|------|----|----------|----------|----------|--|------------------
  2.237 VB S      1 5677.48926    1.12786 5.87809e4     EtOH
  2.623 BB        1   22.23401    1.08555   22.26170    MeCN
  2.782 BB  +I    1   42.04969    1.00000  386.00000    PrCN

Totals without ISTD(s) :            5.88031e4


1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

================================================================================
                          *** End of Report ***
```

Radiopharmacy Dose Sheet for [^{11}C]ER176

Date (mm/dd/yy):	09/11/12	
Batch # (ER176-yymmddxx):	ER176-12091101	
Chemist:	YZ/RX	301-451-3928 (lab)
Radioconcentration (mCi/mL) =	8.28	10:17:39 AM
Specific Radioactivity (mCi/μmol) =	5443.6	10:17:39 AM
ER176 Carrier Concentration (μg/mL) =	0.640	
Total Volume of Formulated [^{11}C]ER176 for Injection (mL) =	9.5	
Maximum Allowable Injection Volume (mL) =	15.6	

Enter slope of calibration curve, M (X= μmol, Y= UV peak area):	8.73106E+08	90% Expected = 453023
Date of calibration curve preparation:	8/21/2012	Expected Area = 503359
		110% Expected = 553695
Enter radioactivity in whole dose vial (mCi):	80.5	
Enter radioactivity in 100 μL aliquot (mCi):	0.832	**Enter End of Synthesis (EOS) Time**
Enter background radioactivity before injection (mCi):	0.004	↓ ↓
Net activity in 100 μL aliquot (mCi) =	0.828	10:17:39 AM
Enter UV peak area of ER176 carrier peak:	132805	
Enter sum of areas of all impurity peaks:	2411	
Percent chemical purity of ER176 =	98.2	
Molecular weight of ER176 =	420.52	
μmol of ER176 in 100 μL aliquot	1.521E-04	
Mass of ER176 in 100 μL aliquot (μg) =	0.06396	
Mass of ER176 equivalent impurity in 100 μL aliquot (μg) =	0.00116	
Maximum allowable injected carrier mass (μg):	10.0	
Maximum allowable injected impurity mass (μg):	1.0	
Maximum allowable injection volume based on carrier mass (mL) =	15.6	Maximum injection volume is the lesser of the two values when calculated from maximum allowable carrier or maximum allowable impurity
Maximum allowable injection volume based on impurity mass (mL) =	86.1	
Second measurement of whole dose vial for half-life calculation (mCi)	63.7	
Time between measurements (min)	7	
Calculated half-life (min)	20.7	

[^{11}C]ER176 Injection: Master Batch Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Approved by: V. W. Pike Initials: VWP Date: 08/28/12

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

Batch # ER176- 120911101 Date: 9/11/12

Reagents/solvents/supplies	Lot/Exp	Purpose	Amount used	Date of Preparation/ Date Opened
Dimethyl sulfoxide, anhydrous	SHBB2380V	Solvent	0.4 mL	8/31/12
Potassium hydroxide, 85%	MKBH 1644V	Reagent Base	1.26 mg	8/6/12
N-desmethyl-ER176	ER169-040711	Precursor	0.59 mg	4/7/11
Ethyl alcohol, USP 200 proof	053213	Cleaning	As required	8/31/12
Sterile Saline for Injection, USP	6003786 / 4/2014	Formulation	10.0 mL	Single Use
Ethanol, dehydrated	1346 / 6/2013	Formulation	0.5 ml	Single Use
Sterile vial 10 mL	11-115-EV / 11/1/2014	Dose vial	1	Single Use
Sterile Millex-GV filter (vent filter, 0.22 µm, 4 mm diameter)	RIJA53440 / 7/2014	Formulation	1	Single Use
Sterile Millex-MP filter (sterilization filter, 0.22 µm, 25 mm diameter)	RIKA64800 / 9/2014	Formulation	1	Single Use
Sterile needle (21 gauge; 2") for sterile filtration, 2 each and [^{11}C]MeI transfer, 2 each	1272662	Formulation	4	Single Use
Sterile needle (20 gauge; 1.5 inches long) for sterile vent	0244285	Formulation	1	Single Use
Water, HPLC grade	52006 / 1/31/13	HPLC mobile phase	As required	9/3/12
Acetonitrile, HPLC grade	DG292	HPLC mobile phase	As required	8/31/12
Methanol, HPLC grade	096964	HPLC mobile phase	As required	9/3/12
Ammonium hydroxide, 1N	2202548 / 02/13	Mobile phase modifier	1 mL / 1 L water	6/1/12
HPLC column (analytical, Phenomenex, C-18 Luna; 10 µm; 4.6 mm × 250 mm)	600405-6	Analytical HPLC	1	8/21/12
HPLC column (semi-prep, Waters, RP-18 XTerra; 10 µm; 7.8 mm × 300 mm)	127I32223119O6	Prep HPLC	1	9/3/12
1 mM ammonium hydroxide (aq)	09-03-2012 / 9/17/12	Prep HPLC mobile phase	1L nominal	9/3/12
MeOH:water, 74:26	09-03-2012 / 9/17/12	Analytical HPLC mobile phase	1L nominal	9/3/12

[^{11}C]ER176 Injection: Master Batch Record

PET Radiopharmaceutical Sciences Section,
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Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Key operation	Initials	Comment/SOP #
Check all gas valves are open and that pressure on regulators are 60, 12 and 22 p.s.i. for nitrogen, helium and hydrogen, respectively	YZ	
6-way valve in NEMA box is set to hot-cell 3 and 3-way valve is on "cryo" position	YZ	
Check gas collection valve is on "fill" position	YZ	
Run prep sequence on GE Microlab Mel box	YZ	
Check flow in RMA, RMB, RMC	YZ	
Check the helium flow rate. Typical flow is 15 to 25 mL/min	YZ	
Check integrity of GE Mel box by running leak check 1	YZ	
Install clean transfer and vent needles at the Trapping station.	YZ	
Verify the ionization chamber with ^{57}Co and ^{137}Cs standards	ccm	
Verify balance using a 10-g NIST traceable weight (acceptable range 9.9–10.1 g)	YZ	Weight: 10.0 g
Record weight of sterile empty vial on QC Form.	YZ	
Remove slider and empty reaction oven	YZ	
Open Synthia AutoRad Software	YZ	
Load Recipe **ER176IND**	YZ	
Enter Reaction Temperature (80 °C) and Time (300 s) when prompted	YZ	
Ensure preparative column switching valve is set to ER176 column and switch is set to "Prep" and install preparative mobile phase	YZ	
Equilibrate the preparative column with 37/63 acetonitrile/1 mM ammonium hydroxide at 8.0 mL/min. Check for leaks. Pressure should be about 3700 p.s.i.	YZ	
Ensure analytical column switching valve is set to ER176 column and switch is set to "Analytical" and install analytical mobile phase	YZ	
Equilibrate the analytical column with 74/26 MeOH/H_2O at 2.5 mL/min. Check for leaks. Pressure should be about 2400 p.s.i.	YZ	
Inject ER176 standard and clean analytical injection port.	YZ	
Test 32Karat Beckman HPLC data acquisition interface box, UV, and PIN diode detector by initiating data collection	YZ	
Connect and wash the product collection line by open the collection valve and flush the Prep mobile phase through the line for at least three minutes.	YZ	
Clean all transfer tubing (20-mL column, HPLC fraction collection line, saline inlet line) with USP ethanol and flush dry.	YZ	
Verify vacuum integrity. Turn on pump; gauge should read approximately 28 inches of mercury (67mBar)	RX	
Connect a 10-mL syringe containing 5% ethanol in saline to end of addition line.	RX	
Fill heating bath with water and set to 80 °C	YZ	

[^{11}C]ER176 Injection: Master Batch Record

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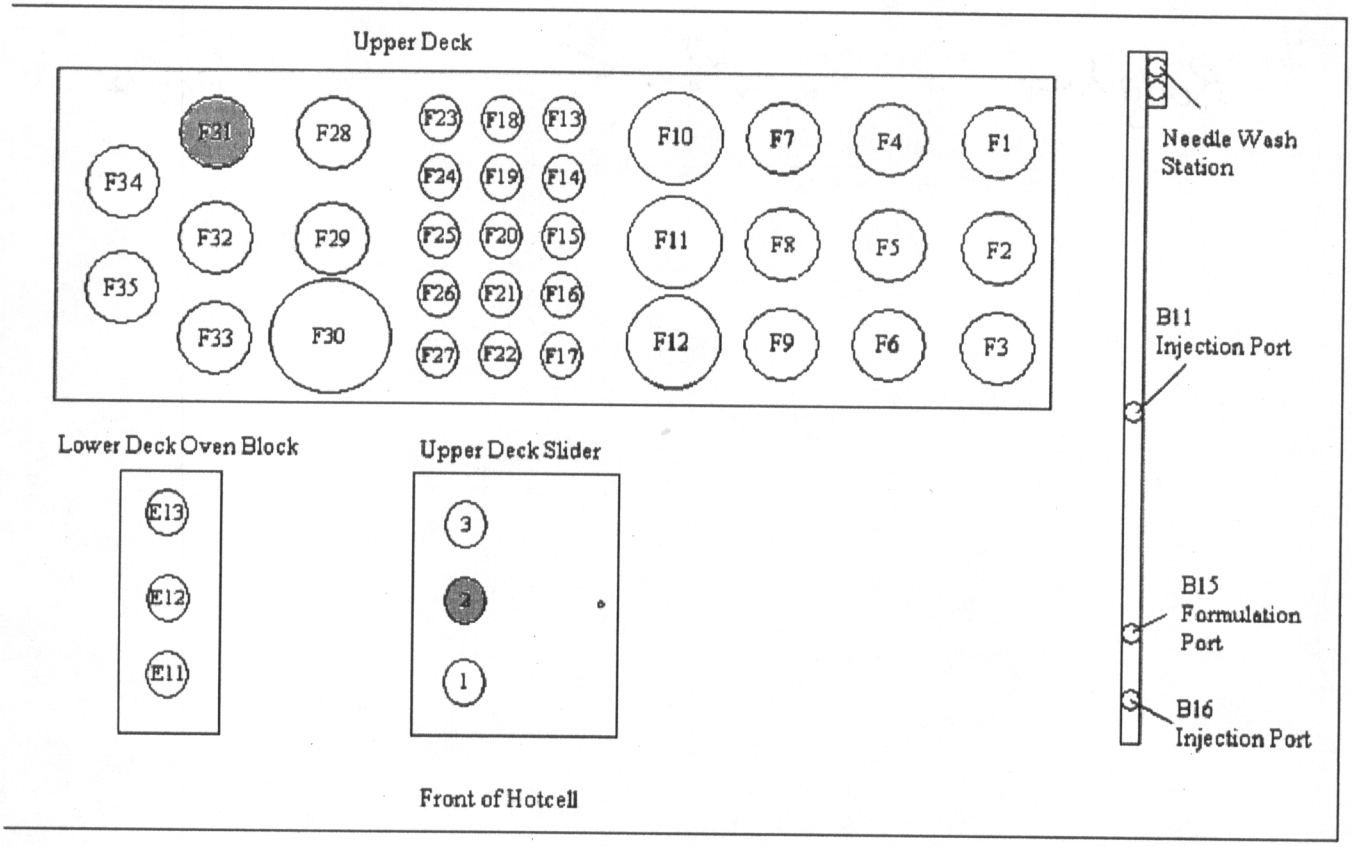
Bethesda, MD 20892

Date of review: 8/28/2012

Check that the dry ice traps are full	Yt	
Record weight of sterile empty vial; prepare and install sterile dose vial unit.	RX	
Check that all waste reservoirs are sufficiently empty	Yt	
Prepare a vial for residual solvent analysis and place in the formulation hot cell	RX	
Prepare the endotoxin unit for operation	RX	
Prepare solution of ER176 precursor (0.6 ± 0.06 mg) / KOH (1.2 ± 0.2 mg) in DMSO (0.4 mL), crimp seal and place in Slider position 2 underneath vent needle when prompted.	RX	

Figure 1: Diagram of Synthia Deck Layout



Position	Tube Type	Solvent / Material
F31	10 mL round bottom	70:30 Absolute Ethanol: HPLC grade Water
Slider Position 2	Reaction Vial	Precursor, KOH, DMSO

[^{11}C]ER176 Injection: Master Batch Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Summary:

Cyclotron, run #	4825 GE2
End of bombardment	9:31
Beam current	45 μA
Bombardment time	40 min
Final formulated product in dose calibrator	80.5 mCi at 10:17:02

Chemist: Rong Xu	Signature: Rong Xu	Date 09/11/12

[^{11}C]ER176 Injection: Quality Control Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Approved by: V. W Pike Initials: VWP Date: 08/28/12
Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Batch # ER176-120911101 Date: 9/11/12

Quality Control Instrument and Materials Verification			
Verification of Ionization Chamber	**Test Data**	**Acceptance Criteria**	**Acceptance Criteria Met**
^{137}Cs source reference ID: 970-31-4 204 µCi on 3/1/2003 Expected radioactivity 163.9 µCi Acceptable Range: 155.7 µCi to 172.1 µCi	^{137}Cs measured: 156.9 µCi	Does measured radioactivity fall within acceptable range (± 5%) for both ^{137}Cs and ^{57}Co?	(Y) / N
^{57}Co source reference ID: 1393-22-20 5516 µCi on 12/01/2009 Expected radioactivity 415 µCi Acceptable Range: 394 µCi to 435 µCi	^{57}Co measured: 405 µCi		
Verification of Analytical HPLC system			
Calibration curve date: 8/21/12 Slope (area/µmol): 8.731 x10^8 Mass injected: 204 Expected peak area: 503359 Acceptable range: 453023 to 553695	Measured peak area: 494384 t_R: 4.9 min	Peak area within ± 10% of expected peak area?	(Y) / N

Post-Production Measurements and Release Tests	
Volume	**Data**
Subtract the weight of the empty dose vial from the full dose vial. Assume the density of the final product is approximately 1 g/mL to determine volume. Weight of full vial: 30.1 g; Weight of empty vial: 20.6 g	Volume of [^{11}C]ER176 Injection: 9.5 mL
Yield	
Measure the activity in the dose vial after removal of the QC sample.	Measured activity: 80.5 mCi at 10:17:02 (EOS)

Test	Test Data	Acceptance Criteria	Criteria Met
pH			
Dispense one to two drops of [^{11}C]ER176 Injection on pH paper.	Measured pH: 5.0	pH measured between 4.0 to 7.5?	(Y) / N
Membrane Filter Integrity			
Attach the syringe filter and needle used for sterile filtration to the compressed air source. Submerge needle tip in water. Pressurize to 45 psi.	Observation: Bubbles / (No Bubbles)	No bubbles observed from the submerged needle tip at 45 psi?	(Y) / N

[^{11}C]ER176 Injection: Quality Control Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Appearance			
Visually inspect the *[^{11}C]ER176 Injection* in the dose vial.	Evaluate appearance	Colorless solution free of particulates?	(Y) / N
Radionuclidic Identity			
Determine the half life experimentally from two time points separated by at least three minutes.	t_1 80.5 mCi at 10:17:02 t_2 63.7 mCi at 10:24:02 Calc. half life: 20.7 min	Experimental half life 20.4 ± 2 min?	(Y) / N
Residual Solvent			
Determine the residual ethanol and acetonitrile in dose by gas chromatography	Ethanol: 5.73×10^4 ng/µL Acetonitrile: 23.55 ng/µL Max. injectable vol: 9.5 mL Acetonitrile in inj. vol: 2.237×10^5 ng	Ethanol ≤ 1×10^5 ng/µL? Acetonitrile ≤ 4.1×10^6 ng?	(Y) / N (Y) / N

Tests Based on HPLC and LC/MS Analysis[1]

Measure the radioactivity of a 100 µL aliquot of *[^{11}C]ER176 Injection:* 832 µCi @ 10:17:39
Measure background radiation before injection: 4 µCi

Radiochemical Identity			
Compare the retention time of *[^{11}C]ER176 Injection* to the ER176 standard retention time.	Standard t_R: 4.9 min Product t_R: 5.108 min	Difference is less than 1.0 min?	(Y) / N
Radiochemical Purity			
Integrate the peaks in the HPLC Bioscan trace. Determine the percent area represented by the product peak.	% Area: 100 %	Greater than 95%?	(Y) / N
Chemical Purity			
Calculate the concentration of carrier and ER176 equivalent impurity in the *[^{11}C]ER176 Injection* from the peak area at 235 nm.	Carrier: 0.640 µg/mL Impurity: 0.0116 µg/mL	Maximum volume contains no more than 10 µg of carrier or 1.0 µg ER176 equivalent impurity.	(Y) / N
Calculate the maximum injection volume as the lesser volume by maximum allowable carrier or maximum allowable equivalent impurity.	Max volume by carrier: 15.6 mL Max volume by impurity: 86.1 mL		
Specific Radioactivity			
Calculate the specific activity of *[^{11}C]ER176 Injection* in units of mCi/µmol	Calculated Specific Activity 5443.6 mCi/µmol	Specific activity ≥ 500 mCi/µmol?	(Y) / N

[1] A calculations worksheet is typically used to perform the calculations required. A copy of the worksheet may be found in Appendix B.

[^{11}C]ER176 Injection: Quality Control Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Bacterial Endotoxin Test (Refer to SOP #QA302)

Reagents/Supplies	Lot # / Expiration Date
EndoSafe Unit ID	1248
Test Cartridge Lot Number	21531651 06/14
Sterile Water Lot Number	60020291 05/14
Pipette Tip Lot	A143326J 1 04/16

	Result	Criteria	Acceptance Criteria Met?
Sample	<2.00 EU/mL Max injectable vol: 9.5 mL EU in injectable vol.: 19.0	≤ 175 EU in total injectable volume	(Y) / N
Sample %CV	0.0%	≤ 25%	(Y) / N
Spike	0.586	Refer to certificate of analysis of each cartridge lot for theoretical value	NA
Spike %CV	3.7	≤ 25%	(Y) / N
Recovery	77%	50 – 200%	(Y) / N

All Quality Control specifications met?	(Y) / N
Chemist: Yi Zhang Signature:	Date: 9/11/12

[^{11}C]ER176 Injection: Post-Release Test Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Approved by: V. W. Pike Initials: VWP Date: 08/28/12
Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Batch # ER176-120911O1 Date: 9/11/12

1. Completed vial label
Attach completed vial label to this form.

[^{11}C]ER176 Injection
Sterile, apyrogenic saline solution for intravenous administration.
Caution: New drug limited by Federal law to investigational use only
NIMH MIB Store at room temperature
Expires 1 h after calibration Half-life of ^{11}C is 20.4 min
Concentration: 8.28 mCi/mL Time: 10:17:39
Activity: 80.5 mCi Volume: 9.5 mL
Date: 9/11/12 Lot #: ER176-120911O1

2. Sterility (Reference SOP# QA302)

Procedure:

After radioactivity has decayed, place the formulated dose in the laminar flow cabinet with one Anaerobic (yellow-cap) and one Aerobic (blue-cap) Bactec test vial. Using aseptic technique, transfer approximately 100 µL formulated dose to each Bactec vial. Record the lot numbers and expiration dates on the sample submission form and take samples and form to the NIH Clinical Center Microbiology Lab.

Results:

Sample form should be returned by Microbiology Lab with test results within 2-4 weeks from date of submission. File results with this form.

Is the *[^{11}C]ER176 Injection* negative for aerobic and anaerobic growth? **Circle one:** (Yes) **or No**

Chemist:	Signature:	Date:
Yi Zhang		9/24/12

REQUEST FOR STERILITY TEST

MIB
Molecular Imaging Branch
-NIMH-

Product: C-11 ER176

Date Submitted for Sterility Testing : 9/12/2012

Lot # : C-11 ER176 -12091101

PHYSICAL AND CHEMICAL CHARACTERISTICS: **NON-RADIOACTIVE MATERIAL**

PRESERVATIVE: **NONE**

REMARKS:

Anaerobic (Yellow) Lot# 2115242, exp. 2013-01-31

Aerobic (Blue) Lot# 2093098, exp. 2013-01-31

RESULTS:

NO GROWTH AFTER 7 DAYS

SIGNATURE: YC DATE: 9-19-12

THIS PRODUCT WAS SUBMITTED FOR STERILITY TESTING BY:

Yi Zhang **301-451 3928**
Bldg. 10, Room B3C355, MSC 1003

IF THERE ARE ANY QUESTIONS OR PROBLEMS CONCERNING THIS PRODUCT, PLEASE CONTACT THE ABOVE INDIVIDUALS.

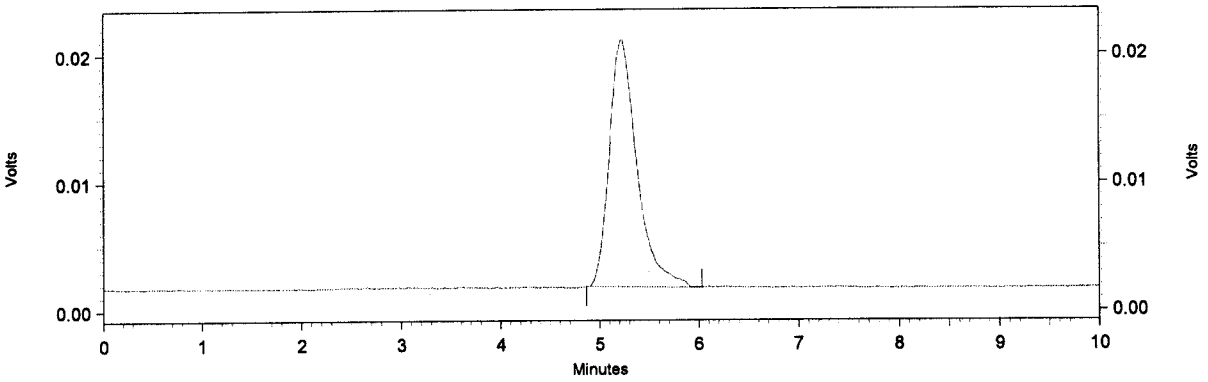
ANALYTICAL HPLC of ER176

Area % Report

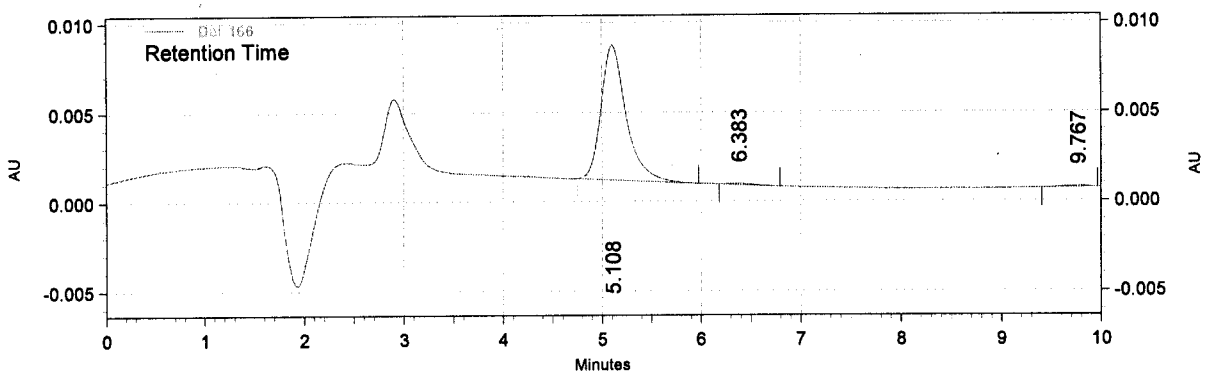
Data File: D:\32Karat\Projects\ER176\Data for IND_Human\Human\ER176-12091101 qc
Method: D:\32Karat\Projects\ER176\Method for IND\ER176 Analytical.met
Acquired: 9/11/2012 10:22:38 AM

HPLC: 74/26 MeOH / water, Luna C18, 4.6 x 250, 10micron
UV = 235 nm, flow rate = 2.5ml/min, detector PMT at 20M, Pressure 2.40kpsi

Bioscan



UV at 235 nm



bioscan Results

Time	Area	Area %	Height	Height %
5.225	375292	100.00	19248	100.00

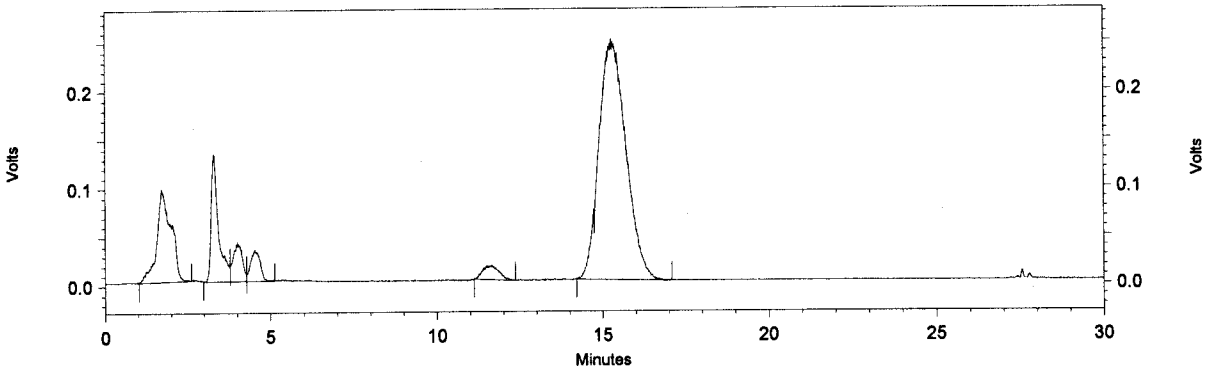
Det 166 Results

Time	Area	Area %	Height	Height %
5.108	132805	98.22	7538	98.07
6.383	1280	0.95	78	1.01
9.767	1131	0.84	70	0.91

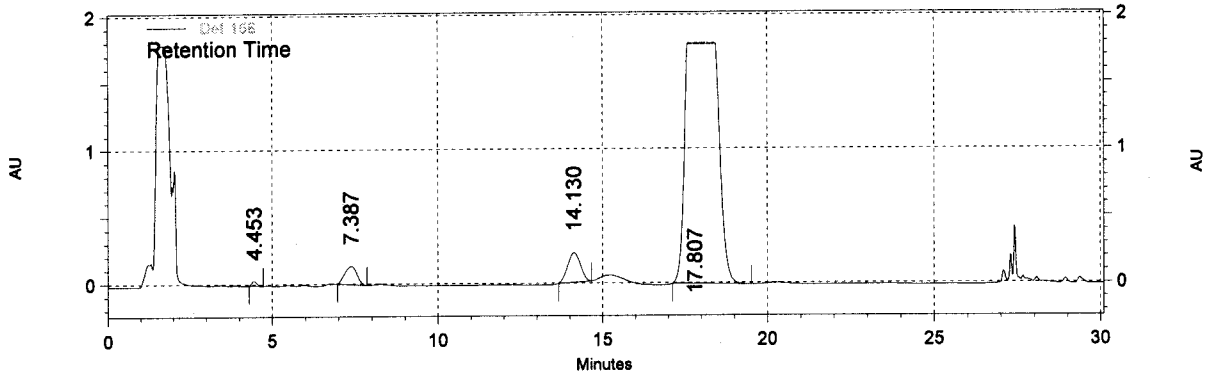
C-11 ER176 via HC#4

Data File: D:\32Karat\Projects\ER176\Data\Human Studies\Validation runs_Sep 2012\ER176-12091101 Prep.dat
Method: D:\32Karat\Projects\ER176\Method\ER176 IND\ER176 Prep.met
Acquired: 9/11/2012 9:51:24 AM
HPLC: Isocratic B/A = 37/63 MeCN/ 1 mM NH4OH; Flow rate = 8.0 ml/min; UV at 235 nm
Waters Xterra, RP18, 10 micron 7.8 mm x300 mm, Pressure 3.7kpsi.

Bioscan



UV 254



Bioscan Results

Time	Area	Area %	Height	Height %
1.725	2895747	13.94	95609	17.01
3.310	2027996	9.77	131145	23.33
4.010	763660	3.68	40477	7.20
4.536	648588	3.12	32459	5.77
11.488	497210	2.39	14329	2.55
15.271	13933743	67.10	248054	44.13

Totals	20766944	100.00	562073	100.00

Det 166 Results

Time	Area	Area %	Height	Height %
4.453	375935	0.28	34270	1.57
7.387	3420954	2.57	136286	6.23
14.130	5936037	4.45	219079	10.02
17.807	123625741	92.70	1797233	82.18

Totals	133358667	100.00	2186868	100.00

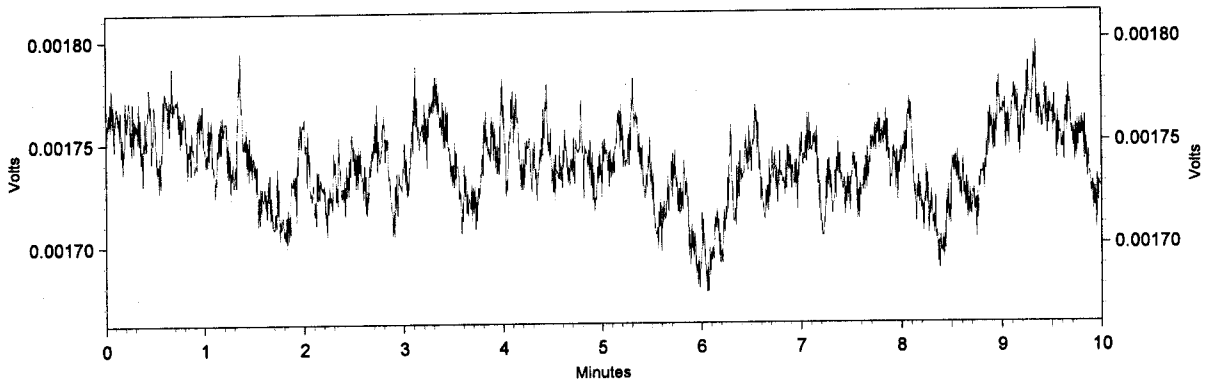
ANALYTICAL HPLC of ER176

Area % Report

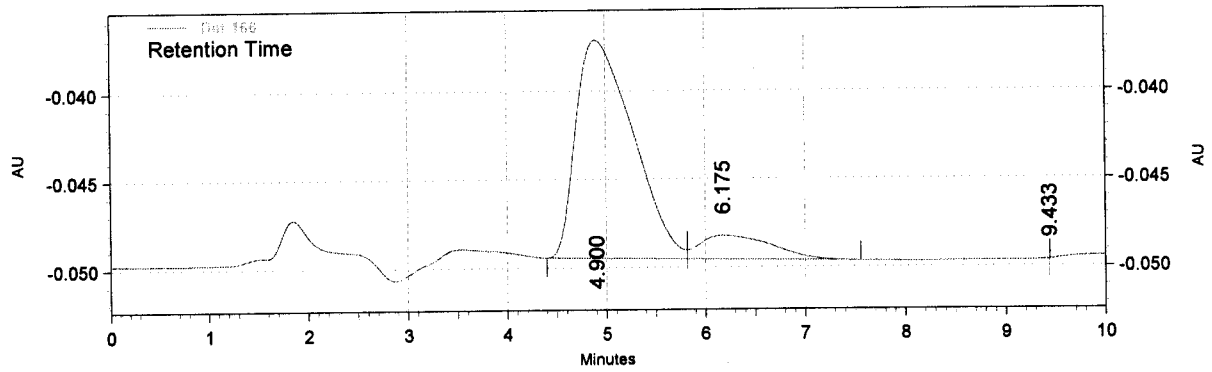
Data File: D:\32Karat\Projects\ER176\Data for IND_Human\Human\ER176-12091101 std
Method: D:\32Karat\Projects\ER176\Method for IND\ER176 Analytical.met
Acquired: 9/11/2012 8:12:08 AM

HPLC: 74/26 MeOH / water, Luna C18, 4.6 x 250, 10micron
UV = 235 nm, flow rate = 2.5ml/min, detector PMT at 20M, Pressure 2.40kpsi

Bioscan



UV at 235 nm



bioscan Results

Time	Area	Area %	Height	Height %

Det 166 Results

Time	Area	Area %	Height	Height %
4.900	494384	88.10	12366	90.20
6.175	66753	11.90	1343	9.80
9.433	0	0.00	0	0.00

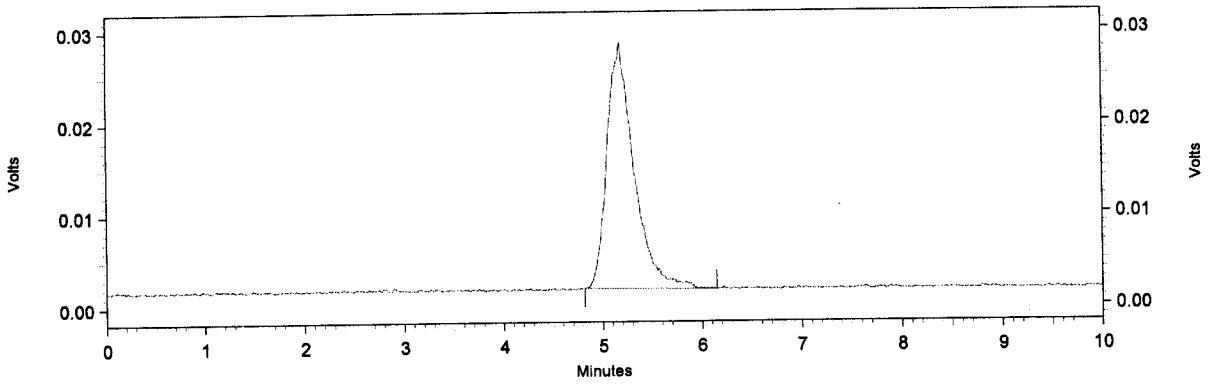
ANALYTICAL HPLC of ER176

Area % Report

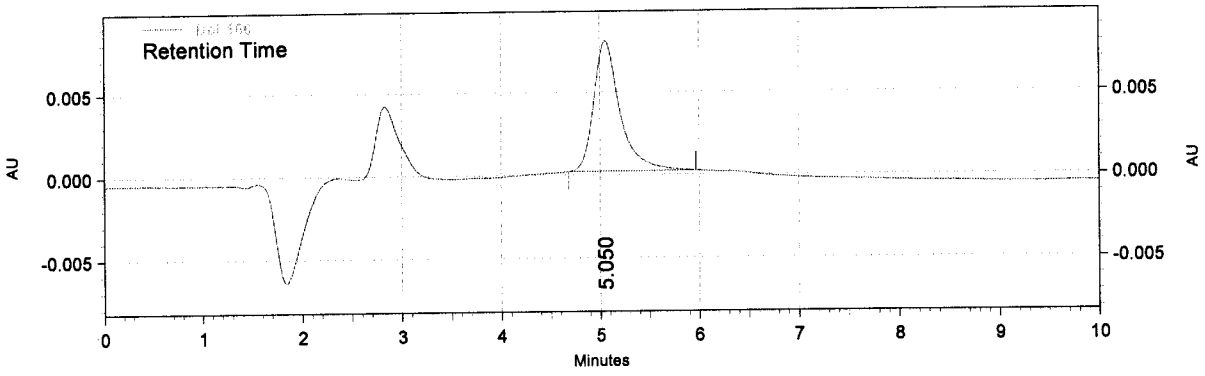
Data File: D:\32Karat\Projects\ER176\Data for IND_Human\Human\ER176-12091101 validation 1hr stability
Method: D:\32Karat\Projects\ER176\Method for IND\ER176 Analytical.met
Acquired: 9/11/2012 11:22:10 AM

HPLC: 74/26 MeOH / water, Luna C18, 4.6 x 250, 10micron
UV = 235 nm, flow rate = 2.5ml/min, detector PMT at 2M, Pressure 2.40kpsi

Bioscan



UV at 235 nm



bioscan Results

Time	Area	Area %	Height	Height %
5.183	505726	100.00	26771	100.00

Det 166 Results

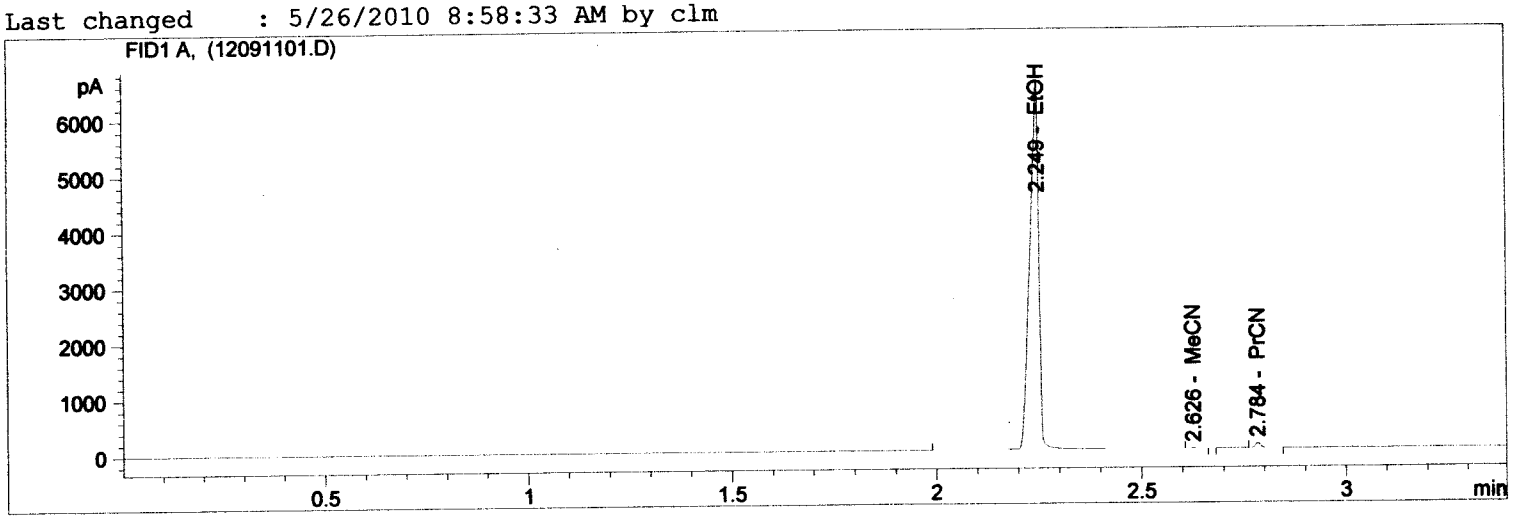
Time	Area	Area %	Height	Height %
5.050	144867	100.00	7860	100.00

Data File C:\CHEM32\1\DATA\ER176\DEF_GC2 2012-09-11 10-34-28\12091101.D
Sample Name: ER176-12091101

Acq. Operator : YZ
Acq. Instrument : Instrument 1
Injection Date : 11-Sep-12, 10:35:47

Seq. Line : 1
Location : Vial 1
Inj : 1
Inj Volume : 1 µl

Sequence File : C:\Chem32\1\DATA\ER176\DEF_GC2 2012-09-11 10-34-28\DEF_GC2.S
Method : C:\CHEM32\1\DATA\ER176\DEF_GC2 2012-09-11 10-34-28\ISPRCN.M (Sequence Method)
Last changed : 5/26/2010 8:58:33 AM by clm



Internal Standard Report

Sorted By : Signal
Calib. Data Modified : 12/13/2007 11:12:57 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 386.00000 [] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
Sample ISTD Information:

ISTD #	ISTD Amount	Name
1	386.00000	PrCN

Signal 1: FID1 A,

RetTime [min]	Type	ISTD used	Area [pA*s]	Amt/Area ratio	Amount	Grp	Name
2.249	VB S	1	9990.68457	1.12786	5.72958e4		EtOH
2.626	BB X	1	4.26712	1.08555	23.55352		MeCN
2.784	BV +I	1	75.91282	1.00000	386.00000		PrCN

Totals without ISTD(s) : 5.73194e4

1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

*** End of Report ***

Instrument 1 9/11/20

Radiopharmacy Dose Sheet for [^{11}C]ER176

Date (mm/dd/yy):	09/11/12	
Batch # (ER176-yymmddxx):	ER176-12091102	
Chemist:	YZ/RX	301-451-3928 (lab)
Radioconcentration (mCi/mL) =	6.92	1:17:50 PM
Specific Radioactivity (mCi/μmol) =	5928.5	1:17:50 PM
ER176 Carrier Concentration (μg/mL) =	0.491	
Total Volume of Formulated [^{11}C]ER176 for Injection (mL) =	8.1	
Maximum Allowable Injection Volume (mL) =	20.4	

Enter slope of calibration curve, M (X= μmol, Y= UV peak area):	8.73106E+08	90% Expected = 453023
Date of calibration curve preparation:	8/21/2012	Expected Area = 503359
		110% Expected = 553695
Enter radioactivity in whole dose vial (mCi):	58.3	
Enter radioactivity in 100 μL aliquot (mCi):	0.694	**Enter End of Synthesis (EOS) Time**
Enter background radioactivity before injection (mCi):	0.002	↓ ↓
Net activity in 100 μL aliquot (mCi) =	0.692	1:17:50 PM
Enter UV peak area of ER176 carrier peak:	101913	
Enter sum of areas of all impurity peaks:	1695	
Percent chemical purity of ER176 =	98.4	
Molecular weight of ER176 =	420.52	
μmol of ER176 in 100 μL aliquot	1.167E-04	
Mass of ER176 in 100 μL aliquot (μg) =	0.04909	
Mass of ER176 equivalent impurity in 100 μL aliquot (μg) =	0.00082	
Maximum allowable injected carrier mass (μg):	10.0	
Maximum allowable injected impurity mass (μg):	1.0	
Maximum allowable injection volume based on carrier mass (mL) =	20.4	Maximum injection volume is the lesser of the two values when calculated from maximum allowable carrier or maximum allowable impurity
Maximum allowable injection volume based on impurity mass (mL) =	122.5	
Second measurement of whole dose vial for half-life calculation (mCi)	50.7	
Time between measurements (min)	4	
Calculated half-life (min)	19.9	

[^{11}C]*ER176 Injection*: Master Batch Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Approved by: V. W. Pike Initials: VWP Date: 08/28/12

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

Batch # ER176-120911102 Date: 9/11/12

Reagents/solvents/supplies	Lot/Exp	Purpose	Amount used	Date of Preparation/ Date Opened
Dimethyl sulfoxide, anhydrous	SHBB2380V	Solvent	0.4 mL	8/21/12
Potassium hydroxide, 85%	MKBH1644V	Reagent Base	1.09 mg	8/6/12
N-desmethyl-ER176	ER169-040711	Precursor	0.63 mg	4/7/11
Ethyl alcohol, USP 200 proof	053213	Cleaning	As required	9/11/12
Sterile Saline for Injection, USP	60037861 04/14	Formulation	10.0 mL	Single Use
Ethanol, dehydrated	1346 I 06/13	Formulation	0.5 ml	Single Use
Sterile vial 10 mL	11-115-EV/11/1/14	Dose vial	1	Single Use
Sterile Millex-GV filter (vent filter, 0.22 µm, 4 mm diameter)	RIJA53440 I 07/14	Formulation	1	Single Use
Sterile Millex-MP filter (sterilization filter, 0.22 µm, 25 mm diameter)	RIKA64800 I 09/14	Formulation	1	Single Use
Sterile needle (21 gauge; 2") for sterile filtration, 2 each and [^{11}C]MeI transfer, 2 each	1272662	Formulation	4	Single Use
Sterile needle (20 gauge; 1.5 inches long) for sterile vent	0244285	Formulation	1	Single Use
Water, HPLC grade	520061 1/31/13	HPLC mobile phase	As required	9/3/12
Acetonitrile, HPLC grade	DG292	HPLC mobile phase	As required	8/31/12
Methanol, HPLC grade	096964	HPLC mobile phase	As required	9/3/12
Ammonium hydroxide, 1N	2202548/02/13	Mobile phase modifier	1 mL / 1 L water	6/1/12
HPLC column (analytical, Phenomenex, C-18 Luna; 10 µm; 4.6 mm × 250 mm)	600405-6	Analytical HPLC	1	8/21/12
HPLC column (semi-prep, Waters, RP-18 XTerra; 10 µm; 7.8 mm × 300 mm)	127I322231190 6	Prep HPLC	1	9/3/12
1 mM ammonium hydroxide (aq)	09-03-20/21 9/17/12	Prep HPLC mobile phase	1L nominal	9/3/12
MeOH:water, 74:26	09-03-20/21 9/17/12	Analytical HPLC mobile phase	1L nominal	9/3/12

[^{11}C]ER176 Injection: Master Batch Record

PET Radiopharmaceutical Sciences Section,
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National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Key operation	Initials	Comment/SOP #
Check all gas valves are open and that pressure on regulators are 60, 12 and 22 p.s.i. for nitrogen, helium and hydrogen, respectively	YZ	
6-way valve in NEMA box is set to hot-cell 3 and 3-way valve is on "cryo" position	YZ	
Check gas collection valve is on "fill" position	YZ	
Run prep sequence on GE Microlab MeI box	YZ	
Check flow in RMA, RMB, RMC	YZ	
Check the helium flow rate. Typical flow is 15 to 25 mL/min	YZ	
Check integrity of GE MeI box by running leak check 1	YZ	
Install clean transfer and vent needles at the Trapping station.	RX	
Verify the ionization chamber with ^{57}Co and ^{137}Cs standards	CCM	
Verify balance using a 10-g NIST traceable weight (acceptable range 9.9−10.1 g)	YZ	Weight: 10.0 g
Record weight of sterile empty vial on QC Form.	YZ	
Remove slider and empty reaction oven	YZ	
Open Synthia AutoRad Software	YZ	
Load Recipe **ER176IND**	YZ	
Enter Reaction Temperature (80 °C) and Time (300 s) when prompted	YZ	
Ensure preparative column switching valve is set to ER176 column and switch is set to "Prep" and install preparative mobile phase	YZ	
Equilibrate the preparative column with 37/63 acetonitrile/1 mM ammonium hydroxide at 8.0 mL/min. Check for leaks. Pressure should be about 3700 p.s.i.	YZ	
Ensure analytical column switching valve is set to ER176 column and switch is set to "Analytical" and install analytical mobile phase	YZ	
Equilibrate the analytical column with 74/26 MeOH/H_2O at 2.5 mL/min. Check for leaks. Pressure should be about 2400 p.s.i.	YZ	
Inject ER176 standard and clean analytical injection port.	YZ	
Test 32Karat Beckman HPLC data acquisition interface box, UV, and PIN diode detector by initiating data collection	YZ	
Connect and wash the product collection line by open the collection valve and flush the Prep mobile phase through the line for at least three minutes.	YZ	
Clean all transfer tubing (20-mL column, HPLC fraction collection line, saline inlet line) with USP ethanol and flush dry.	YZ	
Verify vacuum integrity. Turn on pump; gauge should read approximately 28 inches of mercury (67mBar)	YZ	
Connect a 10-mL syringe containing 5% ethanol in saline to end of addition line.	YZ	
Fill heating bath with water and set to 80 °C	YZ	

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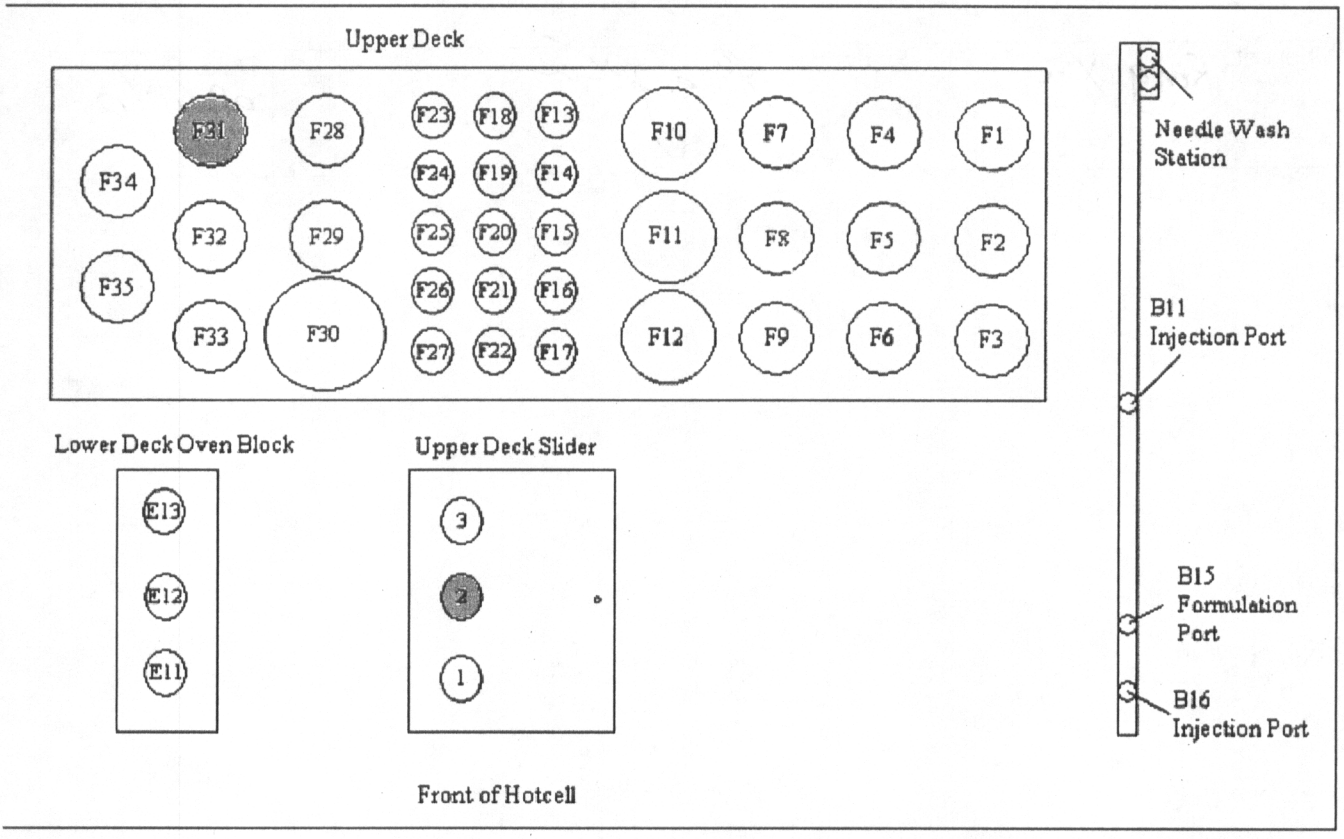
Date of review: 8/28/2012

Check that the dry ice traps are full	Yt	
Record weight of sterile empty vial; prepare and install sterile dose vial unit.	RX	
Check that all waste reservoirs are sufficiently empty	Yt	
Prepare a vial for residual solvent analysis and place in the formulation hot cell	RX	
Prepare the endotoxin unit for operation	RX	
Prepare solution of ER176 precursor (0.6 ± 0.06 mg) / KOH (1.2 ± 0.2 mg) in DMSO (0.4 mL), crimp seal and place in Slider position 2 underneath vent needle when prompted.	RX	

Figure 1: Diagram of Synthia Deck Layout



Position	Tube Type	Solvent / Material
F31	10 mL round bottom	70:30 Absolute Ethanol: HPLC grade Water
Slider Position 2	Reaction Vial	Precursor, KOH, DMSO

[^{11}C]ER176 Injection: Master Batch Record

PET Radiopharmaceutical Sciences Section,
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National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Summary:

Cyclotron, run #	GE-2 4826
End of bombardment	12:30:00
Beam current	45 μA
Bombardment time	40 min
Final formulated product in dose calibrator	58.3 mCi at 13:17:00

Chemist: Rong Xu	Signature: Rong Xu	Date 09/11/12

[^{11}C]ER176 Injection: Quality Control Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Approved by: V. W Pike Initials: VWP Date: 08/28/12
Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Batch # ER176-120911o2 Date: 9/11/12

Quality Control Instrument and Materials Verification			
Verification of Ionization Chamber	**Test Data**	**Acceptance Criteria**	**Acceptance Criteria Met**
^{137}Cs source reference ID: 970-31-4 204 µCi on 3/1/2003 Expected radioactivity 163.9 µCi Acceptable Range: 155.7 µCi to 172.1 µCi	^{137}Cs measured: 156.9 µCi	Does measured radioactivity fall within acceptable range (± 5%) for both ^{137}Cs and ^{57}Co?	(Y) / N
^{57}Co source reference ID: 1393-22-20 5516 µCi on 12/1/2009 Expected radioactivity 415 µCi Acceptable Range: 394 µCi to 435 µCi	^{57}Co measured: 405 µCi		
Verification of Analytical HPLC system			
Calibration curve date: 8/21/12 Slope (area/µmol): 8.731×10^8 Mass injected: 204 Expected peak area: 503359 Acceptable range: 453023 to 553695	Measured peak area: 494384 t_R: 4.90 min	Peak area within ± 10% of expected peak area?	(Y) / N

Post-Production Measurements and Release Tests	
Volume	**Data**
Subtract the weight of the empty dose vial from the full dose vial. Assume the density of the final product is approximately 1 g/mL to determine volume. Weight of full vial: 29.2 g; Weight of empty vial: 21.1 g	Volume of [^{11}C]ER176 Injection: 8.1 mL
Yield	
Measure the activity in the dose vial after removal of the QC sample.	Measured activity: 58.3 mCi at 13:17:00 (EOS)

Test	Test Data	Acceptance Criteria	Criteria Met
pH			
Dispense one to two drops of [^{11}C]ER176 *Injection* on pH paper.	Measured pH: 5.0	pH measured between 4.0 to 7.5?	(Y) / N
Membrane Filter Integrity			
Attach the syringe filter and needle used for sterile filtration to the compressed air source. Submerge needle tip in water. Pressurize to 45 psi.	Observation: Bubbles / (No Bubbles)	No bubbles observed from the submerged needle tip at 45 psi?	(Y) / N

[^{11}C]ER176 Injection: Quality Control Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
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National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Appearance			
Visually inspect the *[^{11}C]ER176 Injection* in the dose vial.	Evaluate appearance	Colorless solution free of particulates?	(Y) / N
Radionuclidic Identity			
Determine the half life experimentally from two time points separated by at least three minutes.	t_1 58.3 mCi at 13:17:00 t_2 50.7 mCi at 13:21:00 Calc. half life: 19.9 min	Experimental half life 20.4 ± 2 min?	(Y) / N
Residual Solvent			
Determine the residual ethanol and acetonitrile in dose by gas chromatography	Ethanol: 5.038×10^4 ng/µL Acetonitrile: 17.696 ng/µL Max. injectable vol: 8.1 mL Acetonitrile in inj. vol: 1.43×10^5 ng	Ethanol ≤ 1×10^5 ng/µL? Acetonitrile ≤ 4.1×10^6 ng?	(Y) / N (Y) / N

Tests Based on HPLC and LC/MS Analysis[1]

Measure the radioactivity of a 100 µL aliquot of *[^{11}C]ER176 Injection*: 694 µCi @ 13:17:50
Measure background radiation before injection: 2 µCi

Radiochemical Identity			
Compare the retention time of *[^{11}C]ER176 Injection* to the ER176 standard retention time.	Standard t_R: 4.90 min Product t_R: 5.092 min	Difference is less than 1.0 min?	(Y) / N
Radiochemical Purity			
Integrate the peaks in the HPLC Bioscan trace. Determine the percent area represented by the product peak.	% Area: 100 %	Greater than 95%?	(Y) / N
Chemical Purity			
Calculate the concentration of carrier and ER176 equivalent impurity in the *[^{11}C]ER176 Injection* from the peak area at 235 nm.	Carrier: 0.491 µg/mL Impurity: 0.0082 µg/mL	Maximum volume contains no more than 10 µg of carrier or 1.0 µg ER176 equivalent impurity.	(Y) / N
Calculate the maximum injection volume as the lesser volume by maximum allowable carrier or maximum allowable equivalent impurity.	Max volume by carrier: 20.4 mL Max volume by impurity: 122.5 mL		
Specific Radioactivity			
Calculate the specific activity of *[^{11}C]ER176 Injection* in units of mCi/µmol	Calculated Specific Activity 5928.5 mCi/µmol	Specific activity ≥ 500 mCi/µmol?	(Y) / N

[1] A calculations worksheet is typically used to perform the calculations required. A copy of the worksheet may be found in Appendix B.

[^{11}C]ER176 Injection: Quality Control Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Bacterial Endotoxin Test (Refer to SOP #QA302)

Reagents/Supplies	Lot # / Expiration Date
EndoSafe Unit ID	1248
Test Cartridge Lot Number	2153165 / 06/14
Sterile Water Lot Number	6002029 / 05/14
Pipette Tip Lot	A143326J / 04/16

	Result	Criteria	Acceptance Criteria Met?
Sample	<2.00 EU/mL Max injectable vol: 8.1 mL EU in injectable vol.: 16.2	≤ 175 EU in total injectable volume	(Y) / N
Sample %CV	0.0%	≤ 25%	(Y) / N
Spike	0.632	Refer to certificate of analysis of each cartridge lot for theoretical value	NA
Spike %CV	4.7%	≤ 25%	(Y) / N
Recovery	83%	50 – 200%	(Y) / N

All Quality Control specifications met?	(Y) / N
Chemist: Yi Zhang Signature:	Date: 9/11/12

[^{11}C]ER176 Injection: Post-Release Test Record

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
Bldg. 10, Rm. B3 C346,
Bethesda, MD 20892

Date of review: 8/28/2012

Approved by: V. W. Pike Initials: V WP Date: 08/28/12
Victor W Pike, Ph.D., Chief, PET, Radiopharmaceutical Sciences, NIMH

Batch # ER176-12091102 Date: 9/11/12

1. Completed vial label
Attach completed vial label to this form.

[^{11}C]ER176 Injection
Sterile, apyrogenic saline solution for intravenous administration.
Caution: New drug limited by Federal law to investigational use only
NIMH MIB
Expires 1 h after calibration
Store at room temperature
Half-life of ^{11}C is 20.4 min
Concentration: 6.92 mCi/mL Time: 13:17:50
Activity: 58.3 mCi Volume: 8.1 mL
Date: 9/11/12 Lot #: ER176-12091102

2. Sterility (Reference SOP# QA302)

Procedure:

After radioactivity has decayed, place the formulated dose in the laminar flow cabinet with one Anaerobic (yellow-cap) and one Aerobic (blue-cap) Bactec test vial. Using aseptic technique, transfer approximately 100 µL formulated dose to each Bactec vial. Record the lot numbers and expiration dates on the sample submission form and take samples and form to the NIH Clinical Center Microbiology Lab.

Results:

Sample form should be returned by Microbiology Lab with test results within 2-4 weeks from date of submission. File results with this form.

Is the *[^{11}C]ER176 Injection* negative for aerobic and anaerobic growth? **Circle one:** (Yes) **or No**

Chemist:	Signature:	Date:
Yi Zhang		9/24/12

G5121556 M 9
DATA MB
00SM0056 NSTCK
09/12/12 10:08 STER
STER LAB

REQUEST FOR STERILITY TEST

MIB
Molecular Imaging Branch
-NIMH-

Product: C-11 ER176

Date Submitted for Sterility Testing : 9/12/2012

Lot # : C-11 ER176 -12091102

PHYSICAL AND CHEMICAL CHARACTERISTICS: **NON-RADIOACTIVE MATERIAL**

PRESERVATIVE: **NONE**

REMARKS:

Anaerobic (Yellow) Lot# 2115242, exp. 2013-01-31

Aerobic (Blue) Lot# 2093098, exp. 2013-01-31

RESULTS:

NO GROWTH AFTER 7 DAYS

SIGNATURE: YC DATE: 9-19-12

THIS PRODUCT WAS SUBMITTED FOR STERILITY TESTING BY:

Yi Zhang **301-451 3928**
Bldg. 10, Room B3C355, MSC 1003

IF THERE ARE ANY QUESTIONS OR PROBLEMS CONCERNING THIS PRODUCT, PLEASE CONTACT THE ABOVE INDIVIDUALS.

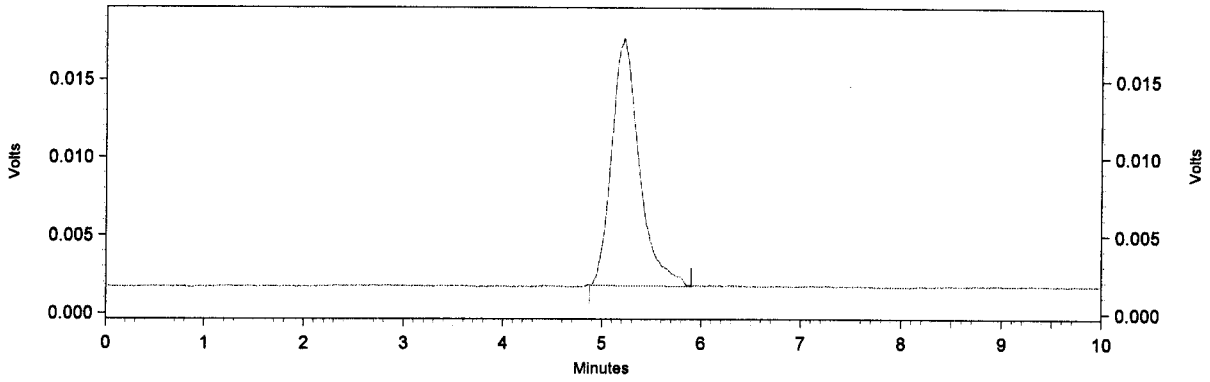
ANALYTICAL HPLC of ER176

Area % Report

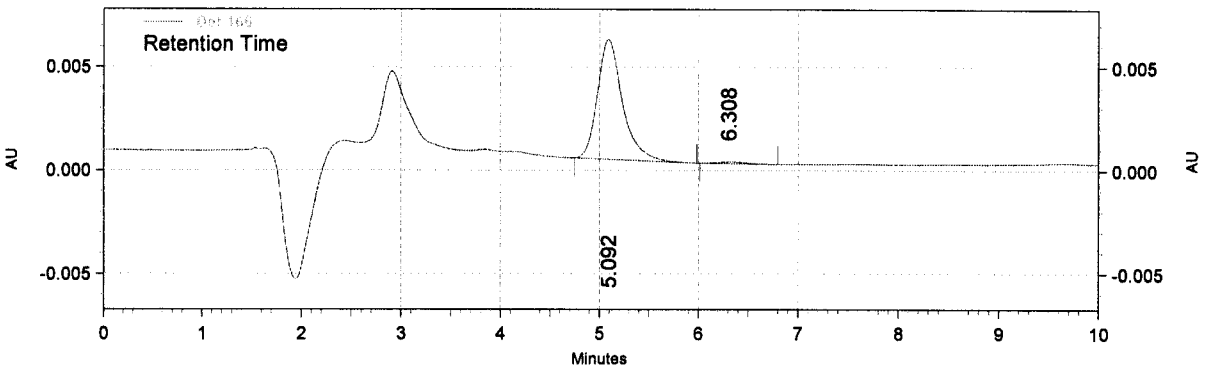
Data File: D:\32Karat\Projects\ER176\Data for IND_Human\Human\ER176-12091102 validation qc
Method: D:\32Karat\Projects\ER176\Method for IND\ER176 Analytical.met
Acquired: 9/11/2012 1:22:40 PM

HPLC: 74/26 MeOH / water, Luna C18, 4.6 x 250, 10micron
UV = 235 nm, flow rate = 2.5ml/min, detector PMT at 20M, Pressure 2.40kpsi

Bioscan



UV at 235 nm



bioscan Results

Time	Area	Area %	Height	Height %
5.216	308174	100.00	15884	100.00

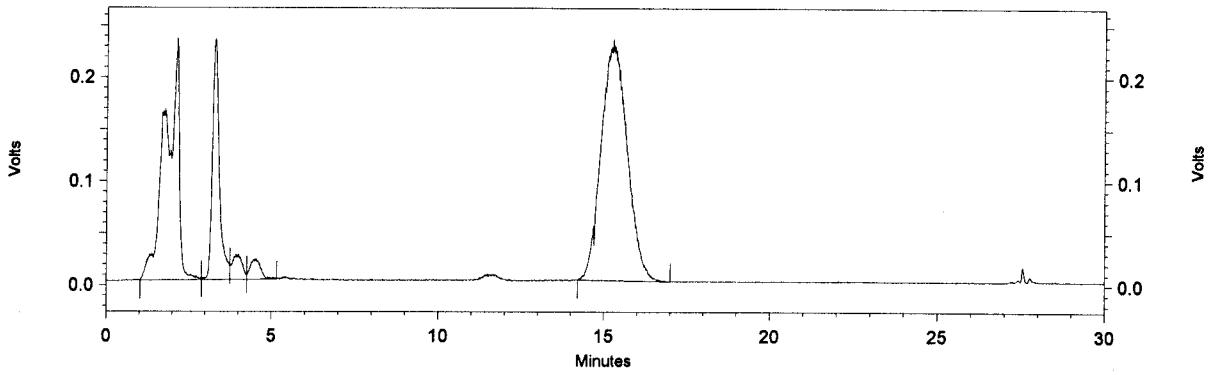
Det 166 Results

Time	Area	Area %	Height	Height %
5.092	101913	98.36	5798	98.56
6.308	1695	1.64	85	1.44

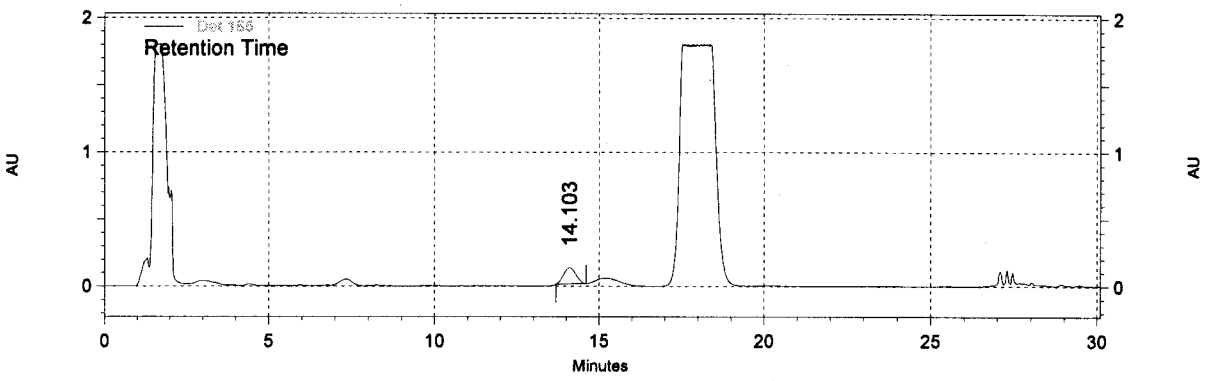
C-11 ER176 via HC#4

Data File: D:\32Karat\Projects\ER176\Data\Human Studies\Validation runs_Sep 2012\ER176-12091102 validation Prep.dat
Method: D:\32Karat\Projects\ER176\Method\ER176 IND\ER176 Prep.met
Acquired: 9/11/2012 12:50:22 PM
HPLC: Isocratic B/A = 37/63 MeCN/ 1 mM NH4OH; Flow rate = 8.0 ml/min; UV at 235 nm Waters Xterra, RP18, 10 micron 7.8 mm x300 mm, Pressure 3.7kpsi.

Bioscan



UV 254



Bioscan Results

Time	Area	Area %	Height	Height %
2.130	6832959	28.77	233102	31.46
3.290	3381278	14.24	231855	31.29
3.923	512085	2.16	24264	3.27
4.496	433182	1.82	19116	2.58
15.271	12587222	53.01	232641	31.40

Totals	23746726	100.00	740978	100.00

Det 166 Results

Time	Area	Area %	Height	Height %
14.103	3159941	100.00	120604	100.00

Totals	3159941	100.00	120604	100.00

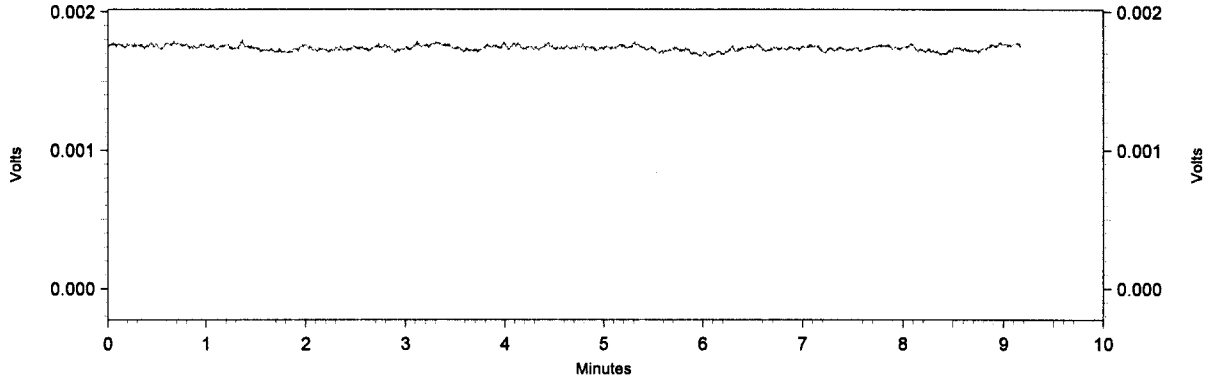
ANALYTICAL HPLC of ER176

Area % Report

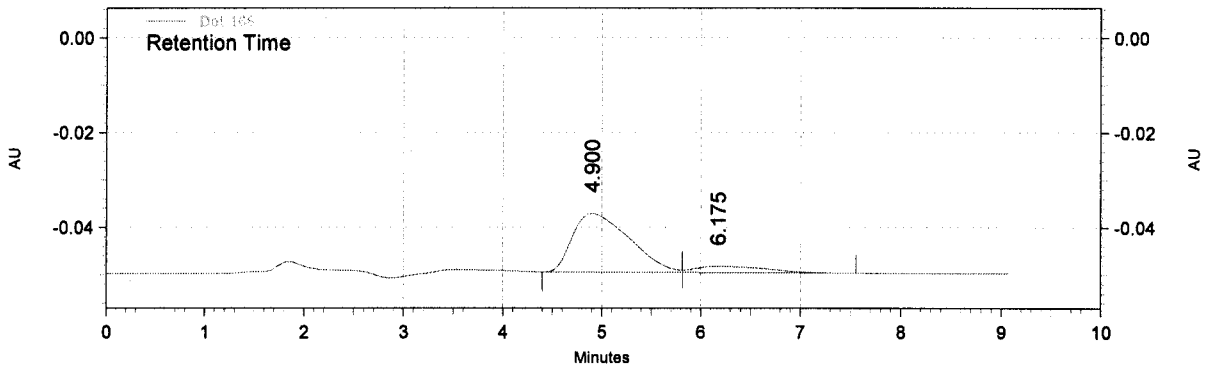
Data File: D:\32Karat\Projects\ER176\Data for IND_Human\Human\ER176-12091101 std
Method: D:\32Karat\Projects\ER176\Method for IND\ER176 Analytical.met
Acquired: 9/11/2012 8:12:08 AM

HPLC: 74/26 MeOH / water, Luna C18, 4.6 x 250, 10micron
UV = 235 nm, flow rate = 2.5ml/min, detector PMT at 20M, Pressure 2.40kpsi

Bioscan



UV at 235 nm



bioscan Results

Time	Area	Area %	Height	Height %

Det 166 Results

Time	Area	Area %	Height	Height %
4.900	494384	88.10	12366	90.20
6.175	66753	11.90	1343	9.80

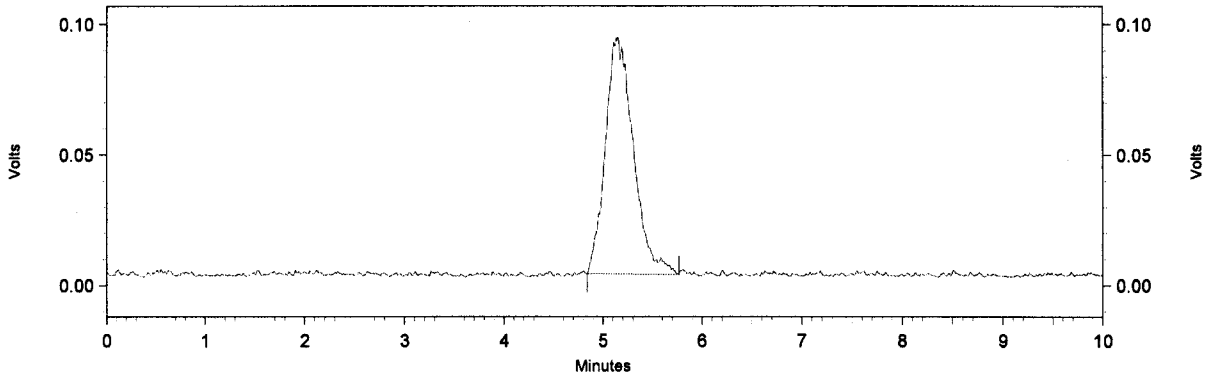
ANALYTICAL HPLC of ER176

Area % Report

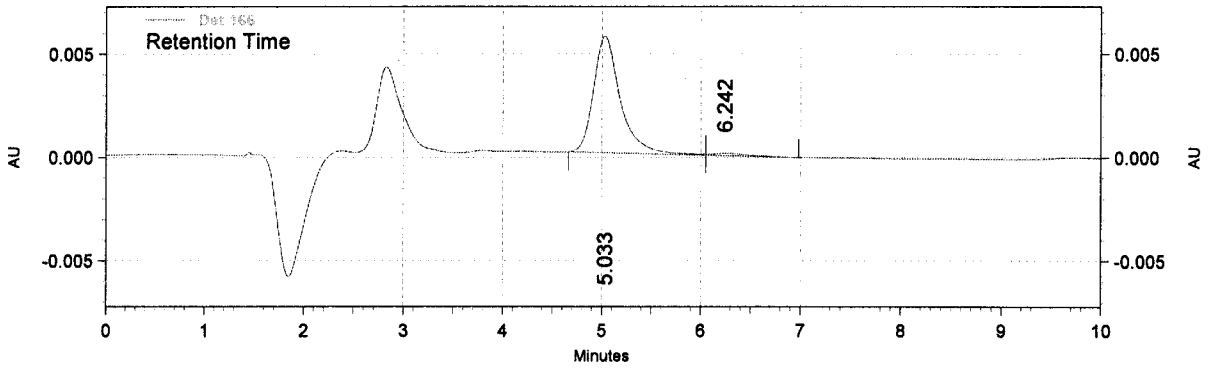
Data File: D:\32Karat\Projects\ER176\Data for IND_Human\Human\ER176-12091102 validation 1 hr stability inj
Method: D:\32Karat\Projects\ER176\Method for IND\ER176 Analytical.met
Acquired: 9/11/2012 2:45:30 PM

HPLC: 74/26 MeOH / water, Luna C18, 4.6 x 250, 10micron
UV = 235 nm, flow rate = 2.5ml/min, detector PMT at 200K, Pressure 2.40kpsi

Bioscan



UV at 235 nm



bioscan Results

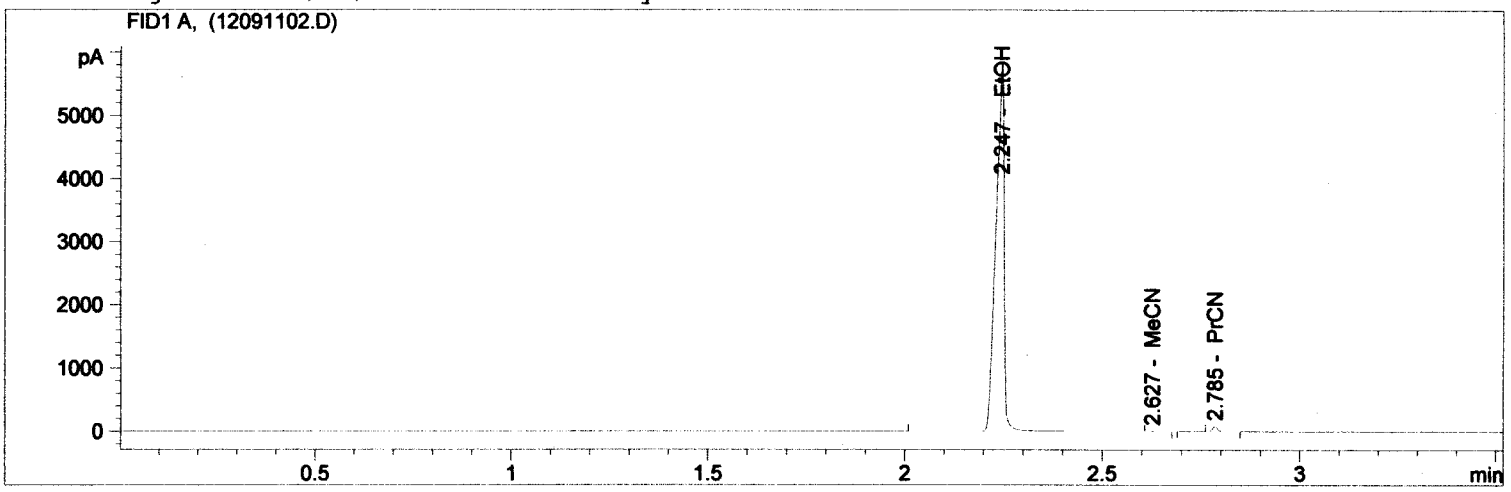
Time	Area	Area %	Height	Height %
5.150	1781886	100.00	90764	100.00

Det 166 Results

Time	Area	Area %	Height	Height %
5.033	105511	97.33	5624	98.01
6.242	2891	2.67	114	1.99

Data File C:\CHEM32\1\DATA\ER176\DEF_GC2 2012-09-11 13-35-24\12091102.D
Sample Name: ER176-12091102

```
Acq. Operator   : RX                                  Seq. Line :   1
Acq. Instrument : Instrument 1                         Location : Vial 1
Injection Date  : 11-Sep-12, 13:36:37                      Inj :   1
                                                     Inj Volume : 1 µl
Sequence File   : C:\Chem32\1\DATA\ER176\DEF_GC2 2012-09-11 13-35-24\DEF_GC2.S
Method          : C:\CHEM32\1\DATA\ER176\DEF_GC2 2012-09-11 13-35-24\ISPRCN.M (Sequence
                  Method)
Last changed    : 5/26/2010 8:58:33 AM by clm
```




```
                        Internal Standard Report

Sorted By             :      Signal
Calib. Data Modified  :      12/13/2007 11:12:57 AM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :    386.00000  []   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
Sample ISTD Information:
ISTD  ISTD Amount   Name
  #
----|------------|------------------------
  1    386.00000   PrCN


Signal 1: FID1 A,

RetTime  Type  ISTD    Area      Amt/Area    Amount    Grp   Name
 [min]         used  [pA*s]       ratio
-------|------|----|----------|----------|----------|--|-----------------
  2.247 VB S      1 8431.45703    1.12786 5.03772e4     EtOH
  2.627 BB X      1    3.07715    1.08555   17.69596    MeCN
  2.785 BB  +I    1   72.86375    1.00000  386.00000    PrCN

Totals without ISTD(s) :            5.03949e4


1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
```

*** End of Report ***