[^{11}C]MeS-IMPY FOR INJECTION: STANDARD OPERATING PROCEDURES

PET Radiopharmaceutical Sciences Section,
Molecular Imaging Branch,
National Institute of Mental Health,
National Institutes of Health,
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Bethesda, MD 20892

Date of review: 09/12/06

List of SOPs for [^{11}C]Mes-IMPY for Injection

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2	Micropore Filter Testing and Drug Product/Filter Compatibility (Bubble Point Test)	SOP # GP102
3	Formulation of [^{11}C]Mes-IMPY for Injection	SOP # GP103
4	Production of [^{11}C]Mes-IMPY for Injection. Part 1: Synthesis and Purification	SOP # MP201
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12	Standard HPLC Calibration Curve of Reference Mes-IMPY	SOP # QA307
13	Acceptance Testing of Desmethyl MeS-IMPY and Reference Compound	SOP # QA308

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SOP # GP101

[^{11}C]Mes-IMPY: Preparation of HPLC Mobile Phases, Precursor Solution, and Reagent Solution

Approved by: _____Initials_____ Date:_____
Victor W Pike, Ph.D., Chief, PET Radiopharmaceutical Sciences, NIMH,

Purpose: 1.) To prepare mobile phases for the HPLC purification of [^{11}C]Mes-IMPY and the QC analysis of [^{11}C]Mes-IMPY for Injection. 2.) To prepare the desmethyl precursor for radiolabeling.

Procedure:

1. The aqueous component for the preparative and analytical HPLC mobile phase (0.1% phosphoric acid) is prepared by adding the following reagents in sequence to a clean 1 L graduated cylinder:

   a) 1.0 ml of 85% phosphoric acid; b) 1 L of water, HPLC grade

2 Stir the buffer with a clean magnetic stir bar. Remove the stir bar with a clean magnetic stir bar retriever. Vacuum filter the 0.1% phosphoric acid through a 0.45 micron nylon filter (Phenomenex AFO-054) and collect the filtrate in a clean 1 L filter flask.

3. Pour the filtrate into a clean 1 L HPLC reservoir bottle. Label the bottle as "0.1% phosphoric acid" and mark the date of preparation. Place this preparative HPLC aqueous mobile phase under hot-cell 3 and place solvent line A1 of 126 pump in the reservoir. The aqueous buffer may be used for one week after the preparation date, provided that it is capped and stored at room temperature when not in use.

4. The isocratic mobile phase for the analytical HPLC system is prepared by adding HPLC grade acetonitrile to 750 mL filtered 0.1% phosphoric acid until the total volume of the unstirred liquid is 1000 mL. The magnetic stir bar is reintroduced into the 1 L graduated

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cylinder and the analytical mobile phase is stirred until homogenous (about 5 min). The contents are transferred to a 1 L HPLC reservoir. Label the reservoir as "25/75 MeCN/0.1% phosphoric acid" and mark the date of preparation. The analytical mobile phase may be used for up to a week after its preparation. Place this analytical HPLC aqueous mobile phase under hot-cell 4 and place solvent line A1 of 118 pump in the reservoir.

5. Precursor solution: 0.5 (± 0.1) mg of desmethyl-Mes-IMPY is weighed out in a 1 mL V-vial and dissolved in 400 µL of anhydrous acetonitrile (Aldrich, # 271004-12 × 100ML). At about 3 min before EOB, 7 µL of a 0.5 M acetonitrile solution of *tert*-butylimino-tris(dimethylamino)phosphorane (BTP) solution is added to the precursor solution via a 5 µL microsyringe (Vici). The acetonitrile precursor solution, containing base, is mixed for about 30 s by mixing the contents of the V-vial using a clean 0.25 mL Hamilton syringe. The 0.5 M *tert*-butylimino-tris(dimethylamino)phosphorane is made within 7 days of production of [^{11}C]Mes-IMPY for Injection. This acetonitrile-base solution is prepared by transferring (via PP/PE syringe) 0.50 mL of pure BTP (Aldrich #445363-5ML) to a clean 5 mL glass vial. To this base solution is added 3.53 mL of anhydrous acetonitrile via PP/PE syringe. The vial is capped, shaken vigorously for 30 s, and then labeled as "0.5 M BTP" and dated.

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SOP # GP102

Micropore Filter Testing and Drug Product /Filter Compatibility (Bubble Point Test)

Purpose: Testing of Micropore filter compatibility.

Approved by: _____Initials_____ Date: _____

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

Procedure:

1. The Millex MP sterile filter that was used for sterile filtration of [^{11}C]Mes-IMPY for Injection is tested by the following method. The intact filter assembly is removed from the sterile dose vial and the 2 in needle attached to the filter is partially submerged in HPLC grade water contained in a clean 25 mL Erlenmeyer flask.
2. The knob on the pressure regulator, used in hot-cell 4 for filter integrity testing, is turned counter clockwise so as to minimize outlet pressure.
3. The transfer line is attached to the 80 p.s.i. house air supply and the air supply toggle valve is flipped to the on position (up).
4. The knob of the pressure regulator is slowly turned clockwise and the pressure gauge is monitored visually. The pressure is brought to 45 p.s.i. If no bubbles are observed at the needle outlet of the sterile filter when the pressure gauge reads 45 p.s.i., then the filter passes the test. The result of the filter integrity test is recorded on the batch record and in the summary section of the QC test form.

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SOP # GP103

Preparation of Formulation Solution

Purpose: Formulation of [^{11}C]Mes-IMPY.

Approved by: _____Initials_____ Date: _____

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

Procedure:

1. Weigh out 20 ± 5 mg of Polysorbate 80, N. F. (J. T. Baker, Cat. # 4117) in a 1-mL V-vial (Waters, P/N 600000671cv).
2. Dissolve Polysorbate 80, N. F. in 0.9 ml of ethanol (USP injection) in 1 ml vial.
3. Pull up ethanol/polysorbate solution and mix it with 10 ml sterile saline.
4. Pull up all the whole content of the above solution in a 10-mL-syringe for formulation.

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SOP # MP201

Production of [^{11}C]Mes-IMPY for Injection. Part 1: Synthesis and Purification

Approved by: _____Initials_____ Date: _____

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

Procedure:

1. Request cyclotron engineer to fill the target with target gas and dump it through waste 1 line of the GE PETtrace Methyl Iodide MicroLab. This is to minimize residual carrier carbon dioxide and should be performed before receiving the day's first batch of [^{11}C]carbon dioxide into the NIMH radiochemistry laboratory.
2. Ensure power is on to all peripheral devices. The iodine quartz column shall not be used more than eight times and shall not be more than two weeks old. Refer to GE PETtrace Methyl Iodide MicroLab manual for preparation of iodine column.
3. Turn valve 6 on, such that the product outlet line of the GE PETtrace is open to Synthia trapping station. Start leak test one on the GE Microlab. Let the helium sweep gas flow to the trapping station and verify that the flow is about 15 mL/min using a hand-held flow meter (Alltech # 9965). Close the end of the product outlet line and check that the system is leak tight. The [^{11}C]iodomethane flow path is considered leak-tight if the RMB flow meter displays zero flow in less than 5 min.
4. Remove used 50 mL pear-shaped flask if present. Clean transfer lines from rotary evaporator fitting and sterile filter transfer line. Specifically, pull up 10 mL of USP grade ethanol in a clean 20 mL polypropylene syringe and push the solvent through the 10 mL Omnifit holding column. Toggle valve 2 on and then off to wash both sides of the transfer

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line. Verify that the stream of HPLC mobile phase run through collection line by toggling collection switch and let it flush the collection line for product fraction for at least 1.0 minutes. Flush the saline addition line with about 5 mL of USP grade ethanol. Flush out the residual solvent in all lines with either house air or Anti-Static Airjet (GC Electronics, # 19-8495-SF). Verify that no liquid is retained in the transfer line to the sterile empty vial. Turn off all 24 V solenoid valves.

5. Pre-load a clean, oven-dried 50 mL pear-shaped collection flask with sodium bicarbonate solution, 8.4% (0.2 mL) on the clean rotary evaporator, to quench acid from mobile phase of the HPLC. Turn on rotary evaporator heat bath and set to 80 ºC. If necessary, add tap water to the heat bath to bring water level to maximum level. Add dry ice-ethanol slurry to rotary evaporator condenser and vacuum pump trap. Verify that round bottom flask is able to spin via remote control and that the rotary evaporator heat bath can be raised and lowered remotely. Place 3.0 mL of 20/80 acetonitrile/water in F8 position of Synthia rack.
6. Run Prep sequence while monitoring RMA, RMB, RMC. Check flow rates and make adjustments to the flow meters if necessary:

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

Use maintenance mode to verify that the following changes to the default parameters are made when the GE PETtrace is rebooted e.g. after power outage: Step 1 (Trap CO_2- 120 seconds); Step 8 (MeI Release- 300 seconds); Prep sequence 4 (Cond. Trap- 180 seconds).

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7. Verify that [^{11}C]carbon dioxide will be directed to hot-cell 3 by confirming that the Valco six-way valve is set to position 3 and the three way valve is on “Cryotrap” position.
8. Upon end of bombardment and verbal confirmation from cyclotron engineer, open [^{11}C]carbon dioxide valve to hot-cell 3 and press run button on GE MeI MicroLab.
9. Load GE_MeSIMPY recipe on Synthia PC, to record distribution of radioactivity during the automated [^{11}C]iodomethane synthesis. Place the reaction vial (Alltech High Recovery vial), with 0.5 $\pm$ 0.1 mg precursor in 0.4 mL acetonitrile and 7 µL 0.5 M BTP solution in acetonitrile, in the middle position of trapping block (position 2). When Step 7 “Methane waste” on GE MeI MicroLab ends (about 11.5 min after EOB), turn on valve 6 to transfer radioactivity to Hot cell 4 and hit “OK” on Synthia program to move down the needle into the reaction vessel.
10. [^{11}C]Methyl iodide produced by GE microlab in hot-cell 3 will be bubbled into the reaction vessel on trapping station of Synthia in hot-cell 4 at 15 mL/min. When the radioactivity is maximized in the reaction vial (about 3−4 min after [^{11}C]methyl iodide release), follow Synthia online instruction to stop gas flow. The reaction vial will be moved by robotic arm (Gilson, Liquid Handler) into an oven and will be heated at 80°C for 5 min. During this time, the preparative HPLC system is prepared to capture the preparative UV and radiation traces that will be generated.
11. The robotic arm will add 3.0 mL of 20/80 acetonitrile/water, pull up the reaction mixture into a syringe, and transfer the mixture into a 5-mL injection loop. The crude radiolabeled product will be purified on a semi-prep HPLC column.
12. A 10 mL sterile, pyrogen-free dose vial is labeled with marker as “C-11 Mes-IMPY” and the protective septum cap is removed. The empty vial is weighed on a validated Acculab Pocket balance (PP-250B) and the weight recorded in the batch record. The certified

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NIMH laminar flow hood contained in Rm 10B3C349 is turned on and the floor of the unit sterilized with isopropyl alcohol. The vial is placed in the laminar flow hood and the top of the septum is wiped with a sterile alcohol swab. Under aseptic technique, a Millex-MP 25 mm 0.2 micron sterile filter is attached to a 2.0 inch 21 gauge sterile disposable needle. The needle assembly is placed through the septum of the sterile dose vial. In a similar fashion the sterile vent filter-needle assembly is placed through the sterile dose vial. A sterile 1 mL polypropylene syringe is fitted with a 1.5 inch 22 gauge sterile needle. This sterile QC sampling syringe is also placed through the septum of the sterile dose vial. Ensure that the vent needle will not touch the final formulated solution. Contact of the vent needle with the solution may cause the solution to escape from the vial. The assembled dose vial is connected to the clean and dry 1/8" Teflon tubing in hot-cell 4 that is connected to the holding column in hot cell 3.

13. Verify that the appropriate HPLC columns are being utilized in hot-cell 4. The preparative HPLC system shall be equilibrated with at least 5 column volumes (*ca*. 60 mL) of the initial mobile phase conditions (recommended flow: 6 mL/min). Upon equilibration the flow rate is reduced to 0.567 mL/min. Similarly, the analytical system shall be equilibrated with at least 5 column volumes (*ca*. 20 mL; recommended flow of 3.0 mL/min) and then the flow reduced to 0.234 mL/min.
14. The column shall be equilibrated with initial phase of the eluate at least 10 min before injection of crude [^{11}C]Mes-IMPY under the gradient HPLC conditions. Load the Prep Mes-IMPY method entitled "Mes-IMPYPrep.met." Verify that the HPLC method is 80% A1 and 20% B1 at 6.0 mL/min at a pressure of about 3.0 kp.s.i.. The wavelength of the absorbance detector is set to 350 nm. Enter the name of the data file for the [^{11}C]Mes-IMPY purification in the following format: (month_date_year_Mesprep#, *e.g.*

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08_04_06_Mesprep1). For preparations intended for human administration, the data path for the saved chromatogram should be D:\32Karat\Projects\Mes-IMPY\Data\Humans*Yr*. Verify that the Bioscan radioactivity detector is set to 2M.

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SOP # MP202

Production of [^{11}C]Mes-IMPY for Injection. Part 2: Collection and Formulation

Approved by: _____Initials_____ Date: _____

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

Purpose: Collection and formulation of [^{11}C]Mes-IMPY for Injection

Procedure:

1. Upon observation of elution reaching 8 min, raise the Swiss boy lift jack, turn on the vacuum pump and verify that the vacuum is below 28 inch of mercury (*ca*. 65 mbar). Turn on spin motor of rotary evaporator.
2. Collect the product fraction (t_R *ca*. 9.3 min) by switching the collect switch in front of hot-cell 4. Verify that the eluate is dripping into the collection flask on the rotary evaporator. If the eluate freezes in the receiving flask as a result of the rapid evaporation of the mobile phase under high vacuum, the frozen solid is removed by transient venting of the vacuum.
3. After finishing collection of product fraction, keep the flask under high vacuum for 1 min 50 s, so as to ensure complete removal of acetonitrile.
4. Open valve to allow air to be admitted into the flask and turn off the vacuum pump. The water bath is then lowered and the formulation solution (10 mL USP sterile saline with 20 mg of polysorbate 80 N. F. and 0.9 mL of USP ethanol) for injection is added remotely to the flask while the flask spins.

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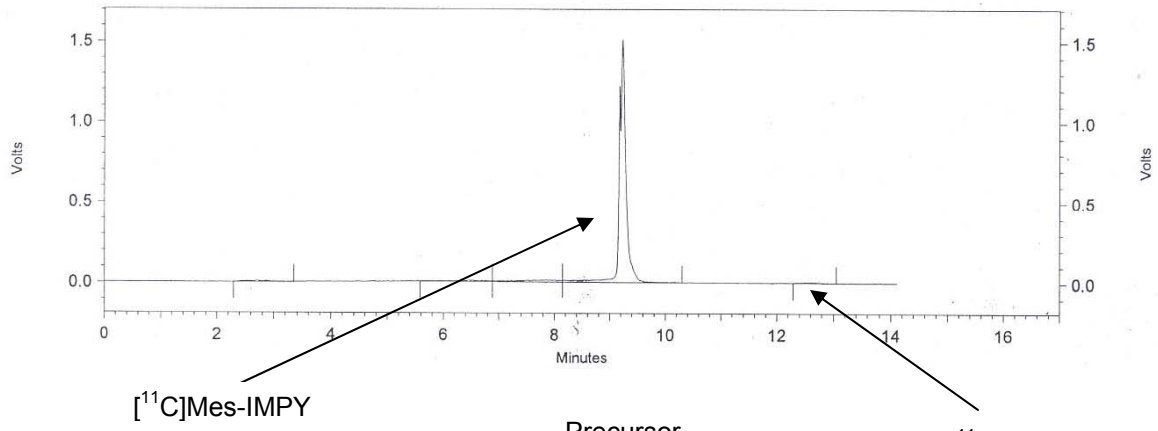
5. The HPLC chromatogram that was generated shall be printed out and included in the batch record. A typical example of an HPLC preparative chromatogram is shown below:

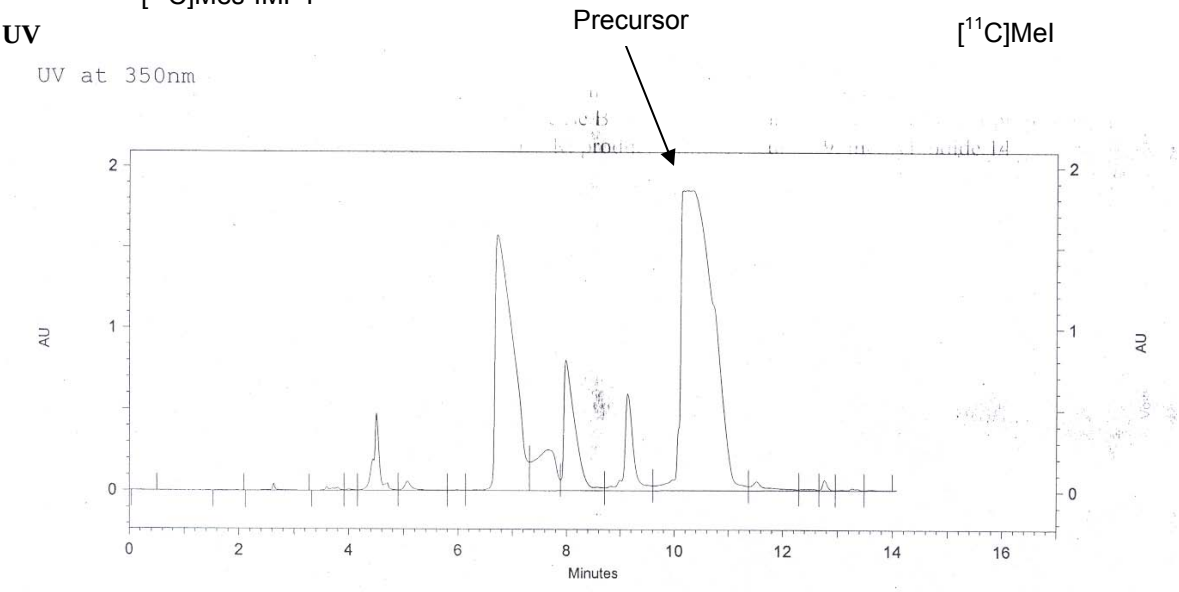
HPLC Data – Preparatory chromatogram

Data File:	D:\32Karat\Projects\MeSIMPY\Data\08_24_2006 mesimpy prep
Method:	D:\32Karat\Projects\MeSIMPY\Method\MeSIMPY IND_GRAD20to50.met
Acquired:	8/24/2006 2:43:07 PM
Printed:	08/24/2006 03:19:05 AM
HPLC:	Gradient MeCN/0.1% phosphoric acid at 6 mL/min C-18 Phenomenex Luna semiprep. 350 nm.

Bioscan-Radioactivity



UV



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6. The switching valve that directs the column eluate through the UV and radioactivity detectors is changed to the analytical position. Also, the Bioscan radioactivity detector setting is switched to 20K.
7. The formulated [^{11}C]Mes-IMPY is transferred to the 10 mL sterile dose vial via actuation of valve 2 and pulling back on the plunger of the 60 mL syringe. Upon complete transfer of the 10 mL volume from the round bottom flask to the holding column, valve 2 is turned-off and the 60 mL syringe plunger is pushed inward. This will direct the formulated [^{11}C]Mes-IMPY to the sterile filter and into the 10 mL sterile dose vial. A QC sample of [^{11}C]Mes-IMPY for Injection is removed from the dose vial (see Document 5, SOP # QA 301).
8. After release of [^{11}C]Mes-IMPY for Injection, wash out the preparative column by putting A1 solvent line in clean HPLC water and running prep_column_wash_Mes.met. After quality control analysis of [^{11}C]Mes-IMPY for Injection (See Document 5, SOP #QA 303), flush out the analytical column at the flow rate of 2 mL/min for at least 20 min with 50: 50 = acetonitrile- HPLC grade water. Clean-up all used disposables in hot-cell 4. Fill out all forms relating to the production/quality control of the batch of [^{11}C]Mes-IMPY for Injection.

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SOP # QA301

Post-filtration Sampling for QC

Approved by: _____Initials_____ Date: _____

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

Purpose: Sampling of [^{11}C]Mes-IMPY for QC

1. The 1 mL sterile, sampling syringe previously inserted in the 10 cc dose vial containing [^{11}C]Mes-IMPY is used to remove a 1.0 mL sample. One drop (*ca*. 0.050 mL) of this sample is dispensed onto a pH paper to measure pH of the [^{11}C]Mes-IMPY for Injection (see QC form, document 3). The remainder of the sample is divided into several different glass vessels. About 0.3 mL is placed in an open-top 1 mL v-vial. This sample will be utilized for HPLC analysis and will be used to assess/measure radioconcentration, radiochemical purity, radiochemical identity, specific radioactivity and chemical purity. The remaining 0.65 mL sample of [^{11}C]Mes-IMPY for Injection is placed in a pyrogen-free dilution tube and will be utilized for pyrogen testing and any additional QC testing that is not required as a condition of release of [^{11}C]Mes-IMPY for Injection (*e.g.* LC-MS, residual volatile solvents assay).
2. SOPs for the pH, radioconcentration, radiochemical purity, radiochemical identity, specific radioactivity and chemical purity tests should be followed and can be found in the QC and Radiopharmacy forms (Document 3 and 4 of CMC section, respectively). The more detailed SOP for filter integrity testing and testing of residual volatile organics (performed

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for the three validating runs of [^{11}C]Mes-IMPY for Injection), can be found in Document 5, SOP # GP102 and SOP # QA302, respectively.

3. After removal of the 1 mL QC sample, the dose vial is weighed on the Acculab balance, and the weight is recorded on the batch record so as to determine the final volume of the [^{11}C]Mes-IMPY for Injection. The radioactivity contained in the sterile dose vial is measured in the ionization chamber of hot-cell 4. The amount of [^{11}C]Mes-IMPY for Injection (in mCi) and time are recorded on the batch record.

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SOP # QA302

Analysis of Organic Residues in [^{11}C]Mes-IMPY for Injection by Gas Chromatography

Approved by: _____Initials_____ Date: _____

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

Purpose: to analyze for volatile solvent residues in [^{11}C]Mes-IMPY for Injection

1. Responsibility
   1.1 It is the responsibility of the Chief of PRSS to ensure that personnel are trained in this procedure.
   1.2 It is the responsibility of personnel to adhere to this approved SOP.
   1.3 Procedure adheres to GMP/GLP guidelines.
2. Scope - QC testing of volatile organic solvents in NIMH produced PET radiopharmaceuticals.
3. Reference Document(s)
   3.1 6850A GC User Information
   3.2 Installation, operation and maintenance manual for hydrogen generator (Model H2-90, Parker Balston; and Bulletin TI-H2-90C)
4. Safety Precautions
   4.1 Radiation Safety – ALARA (As Low as Reasonably Acceptable)
   4.2 Chemical laboratory safety
5. Materials and equipment
   5.1 Agilent 6850 GC with flame ionization detector (FID)
   5.2 Agilent 6850 series autosampler

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5.3 J & W DBWAX column, 30 m (l) × 0.25 mm (id) × 0.25 μm (film thickness) (Alltech, part # 122-7032)

5.4 Acquisition and data processing software: GC Chem Station (version: Rev. A.09.03 [1417])

5.5 Inlet liner: split inlet glass liner with glass wool packing (Agilent part number, 5183-469119251-60540)

5.6 Parker Balston H2-90 Hydrogen Generator

5.7 High purity grade (99.995 %) compressed helium (Roberts Oxygen, cat. no. R 102 F3)

5.8 In-house air purified by Parker Balston Zero Air Generator, Model 75-83NA

5.9 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)

5.10 Pipetman 200 μL pipette (NIH stock # 6640-02-032-1955)

5.11 386 ppm internal standard aqueous solution of propionitrile prepared via 1 in 10 dilution of a 3860 ppm solution (0.5 mL propionitrile diluted to 100 mL mark with water).

6. GC system configuration.

6.1 Injection port: split sample injection split ratio of 20: 1, 250 ˚C

6.2 Carrier gas: Helium; 2 mL/min

6.3 Column temperature gradient: column temperature is initially operated at 50˚C and held at this temperature for 1 min, and then increased to 150˚C at a rate of 20˚C /min. The temperature is held at 150˚C for 0.5 min and then increased to 220˚C at a rate of 50˚C/min. After 3 min at 220˚C the column temperature is returned to starting temperature of 50˚C.

6.4 Detector: FID with hydrogen at 40 mL/min and air at 450 mL/min. Helium make-up flow: 45 mL/min. Detector at 250 ˚C.

6.5 Autosampler: 10 μL syringe. Sample injection volume: 1 μL.

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6.6 Needle/Syringe wash: four times before injection of sample and two times after injection.

7. Procedure before data acquisition**.** Verify that paper is loaded in the printer and the power is turned on to the printer. Ensure that the water level in the H2-90 hydrogen generator is above the lower limit. Otherwise, pour in 18 MΩ water into the reservoir until the level is just below the upper limit of water reservoir. Check the hydrogen pressure is about 28 p.s.i. Check helium pressure is *ca*. 60 p.s.i. on main cylinder gauge. Make sure that the solvents A and B (in the autosampler tray) for rinsing injection syringe needle are filled with deionized (18 MΩ) water. With a 200 μL Pipetman, pipette 50 μL of [^{11}C]Mes-IMPY for Injection (test sample) into a glass insert housed in a GC autosampler vial. Discard the radioactive pipette tip in radioactive waste. Add 50 μL of the internal standard solution of propionitrile (386 ppm) to the test sample. Cap the autosampler vial with septum. Tap the bottom of the autosampler vial to remove air bubbles. Place autosampler vial in autosampler rack and note rack position. On the GC control panel, use up or down arrow buttons to verify that FID is lit and the background signal is about 5.

8. Data acquisition of [^{11}C]Mes-IMPY test sample spiked with internal standard. Double click “Instrument 1 online” on the desktop. Select Method and Run Control from drop down menu bar below the file menu bar. Select ISPRN.M from drop down menu box on the right side of Method and Run Control Box. Go to sequence on the main menu and select sequence parameter. Type in subdirectory for radiopharmaceutical that will be tested (i.e. Mes-IMPY). Select Sequence Table from Sequence drop down menu. Enter 1 for the number of injections per sample and location of the injection. Enter the sample name and verify method name is ISPRCN. Enter file name (no more than 7 characters), and injection volume (1.0 μL). Verify that the carousel icon is showing on the left of the computer monitor. When the system shows a ready sign (above start button), hit the start button. To view on line signal, go to view on the main menu bar and select on line signal/signal window 1. After 3.5 min, the GC chromatogram and report can be printed out by hitting the stop button followed by the print button that appears under the report displayed on the monitor. If the report is not printed out, one can print the report by opening “Instrument 1 offline”. On the left side of the screen, scroll the drop down menu to “data analysis”. Under the file menu, scroll to “load signal”. Find the file name in the appropriate

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subdirectory on the C drive (e.g. C:\. . . \data\Mes-IMPY). After loading the signal, scroll down to "print preview . . . report" that is found in the file menu. The chromatogram and report should appear on the computer screen and this can be printed out by hitting the print button on the bottom of the report page. Attach the chromatogram and report to the quality control form. For a sample to pass the residual solvent test, the amount of acetonitrile (MeCN, t_R *ca.* 2.67 min) should be less than 400 ng and the amount of ethanol (EtOH, t_R *ca.* 2.27 min) should be less than 100,000 ng based on a 1 μL injection.

9. Post-run method. Remove all samples and label radioactive samples. Download the method Default.M that is used to maintain the oven temperature at 150 ˚C when GC is idle.

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Date of review: 09/12/06

SOP #QA303

Analytical HPLC QC Method

Approved by: _____Initials_____ Date: _____

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

Purpose: to perform analytical HPLC QC on [^{11}C]Mes-IMPY for Injection

Procedure; Part 1: Analytical HPLC QC Method: Pre-EOB Protocol

1. Ensure that the 4-port switching valve is set to analytical system. Before analysis of the [^{11}C]Mes-IMPY, the analytical column (C-18 Luna; Phenomenex; catalog # 00G-4094-E0) is equilibrated with the analytical mobile phase (25: 75 acetonitrile: 0.1% aqueous phosphoric acid; see Document 5, SOP # GP101 for preparation) for at least 8 min with flow set at 3.0 mL/min and the UV detector at 350 nm.

2. The analytical method, analytical_Mes-IMPY.met, is downloaded (path D:\32Karat\projects\Mes-IMPY\analytical_Mes-IMPY.met). A standard injection of Mes-IMPY (100 ng) is performed at least 40 min before the end of synthesis of [^{11}C]Mes-IMPY for Injection. A standard solution of Mes-IMPY (100 ng; 3.53 x 10^{-4} μmol) is injected on the analytical column (file name format: month_date_year_Mes-IMPY_100ng_inj#). The UV chromatogram is recorded for at least 5 min and the peak area for Mes-IMPY is recorded. The peak area should be ± 10% of the expected peak area based on a 5-point calibration curve. The expected retention for Mes-IMPY is about 4.0 min. If more than one batch of [^{11}C]Mes-IMPY for Injection is being prepared in a single day, then a standard solution of

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Mes-IMPY need only to be injected on the analytical column before the first production run of the day.

3. After injection of the Mes-IMPY standard, 2 mL of USP ethanol is pulled-up in a 5 mL glass Hamilton syringe and the ethanol is loaded on the analytical HPLC loop. The ethanol is injected to flush out residual Mes-IMPY in the HPLC injector while passing the solvent through the column at 3.0 mL/min for at least 10 min (no data collection necessary). All QC chromatograms should be printed out and included in the QC form that is included in the batch production record.

Procedure. Part 2: Analytical HPLC QC Method: Post EOS Protocol

1. An aliquot (0.1 mL) of the 1.0 mL fraction that was obtained as described in Document 5, SOP # QA301 is used for the following tests: radioconcentration, radiochemical purity, radiochemical identity, specific radioactivity and chemical purity. Details of the exact procedures to be used for these tests can be found on the QC form (Document 3). The results of all QC tests are recorded on the QC record.

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SOP #QA304

Release of [^{11}C]Mes-IMPY for Injection

Approved by: _____Initials_____ Date: _____

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

Purpose: to clarify tests that are to be completed before release of [^{11}C]Mes-IMPY for Injection and labeling procedure

Procedure:

1. The following tests must be completed and the batch of [^{11}C]Mes-IMPY for Injection meet all specifications before release from the NIMH production site: a) radiochemical identity; b) radiochemical purity; c) chemical purity; d) radioconcentration; e) pH; f) specific radioactivity; g) membrane filter integrity; h) appearance test. The results of these tests are tabulated in the QC form.

2. If the batch of [^{11}C]Mes-IMPY for Injection meets all acceptance criteria for the above tests (see CMC section or QC form for criteria), then two labels are filled out completely. Radioactivity is recorded on the label as the product of radioconcentration (mCi/mL) and volume (mL) at the calibration time. One label is attached to the 10 mL dose vial containing the [^{11}C]Mes-IMPY for Injection; the second label is attached to the radiopharmacy form. The QC chemist signs and dates the QC form authorizing release from the production/quality control area. A sample of a duplicate blank dose vial label is provided below:

[^{11}C]MeS-IMPY FOR INJECTION:
STANDARD OPERATING PROCEDURES

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[^{11}C]Mes-IMPY for 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:_____mCi/mL Activity:_____mCi Volume:_____mL Calib. Date:_____ Time:_____ Lot #:_____	**[^{11}C]Mes-IMPY for 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:_____mCi/mL Activity:_____mCi Volume:_____mL Calib. Date:_____ Time:_____ Lot #:_____

[^{11}C]MeS-IMPY FOR INJECTION: STANDARD OPERATING PROCEDURES

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SOP #QA305

Post-release QC Tests {Bacterial Endotoxins (LAL Test) and Sterility Test}

Approved by: _____Initials_____ Date: _____

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

Purpose: to test the pyrogenicity and sterility of [^{11}C]Mes-IMPY for Injection

Procedure

1. Quality control tests for sterility and bacterial endotoxins (LAL test) are to be performed on each batch of [^{11}C]Mes-IMPY for Injection. However, owing to the time needed to perform these tests, the [^{11}C]Mes-IMPY for Injection may be released before test completion.

2. Bacterial endotoxin testing should commence within 24 h of EOS of [^{11}C]Mes-IMPY for Injection. When practical, a sample for sterility testing should be submitted one day after [^{11}C]Mes-IMPY production/human administration. In the case of [^{11}C]Mes-IMPY for Injection that was produced on a Friday or a workday preceding a Federal holiday, sampling and submission of the sterility test sample should proceed on the next Federal workday. Test results and reports should be included on the Radiopharmacy form (Document 4 of CMC section) that provides standard operating procedures for performance of these two tests.

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SOP #QA306

Annual QC Test for Radionuclidic Identity

Approved by: _____Initials_____ Date: _____

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

Purpose: to test the identity of radionuclide in [^{11}C]Mes-IMPY for Injection

Procedure:

1. Follow the SOP as outlined in Document 6, "Standard Operating Procedure and Results from Annual Radionuclide Identity Test for Carbon-11" and record data as required. The experimentally determined half-life of a batch of [^{11}C]Mes-IMPY for Injection should be between 18 and 22 min. Include a copy of the report in the batch production record of the first validating production run of [^{11}C]Mes-IMPY for Injection. Annually thereafter radionuclidic identity should be tested on a batch of [^{11}C]Mes-IMPY and a copy of this updated radionuclidic purity report should be placed in the batch production record of the first validating production run of [^{11}C]Mes-IMPY for Injection.

[^{11}C]MeS-IMPY FOR INJECTION: STANDARD OPERATING PROCEDURES

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SOP # QA307

Standard HPLC Calibration Curve of Reference Mes-IMPY

Approved by: _____Initials_____ Date: _____

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

Purpose: to generate a calibration curve to determine the mass of the carrier in [^{11}C]Mes-IMPY for injection

A. Stock solution of reference Mes-IMPY

All data should be recorded on Document 9, data record of preparation of standard solution of Mes-IMPY. See Document 9 for detailed procedures. In brief, weigh an accurate mass of Mes-IMPY (*ca*. 10 mg) in a tared 25 mL flask on a validated balance. Dissolve in DMSO in a 10 mL-volumetric flask for QC. Mix thoroughly for several minutes. The stock solution is dispatched into small vials (20 × 1.2 mL). When not in use, store at − 20 °C in the freezer.

Concentration of solution = ~ **0.4 µg/µL**

B. Diluted Mes-IMPY standard solution

From the stock solution make a dilution 1: 100

- Example: take 1000 µL of stock solution. Dilute with solvent (DMSO) to the 9 mL mark of a clean volumetric flask (10 mL size). Mix well to ensure homogeneity. The stock solution is dispatched into small vials (90 × 0.1 mL). When not in use, store at − 20 °C in the freezer. When needed, warm a vial to RT and add 0.9 mL HPLC water into the vial and vortex it.

  Concentration: ~ **4 ng/µL**

  µmol of Mes-IMPY (M.Wt. = 283.39) in this dilution:

  4 ng/283.39 = 1.41 x 10^{-5} µmol in 1.0 µL

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C. Procedure for obtaining standard curve

1. Open the Beckman 32 karat software and select the instrument “Analysis System” to start HPLC system.
2. Set the analytical HPLC System (Beckman Coulter) in working conditions and keep flow for 30 min for column equilibration.

HPLC System conditions:
Column: Luna C-18 (10 µ; 4.6 × 250 mm; Phenomenex)
Mobile Phase: Acetonitrile-0.1% aqueous phosphoric acid (25: 75 v/v)
Flow Rate: 3.0 mL/min.
UV Wavelength: 350 nm

3. Select the method “Analytical_Mes-IMPY” in method box and check the instrument setup on the “Instrument Setup” box, then close box.
4. Use the diluted Mes-IMPY standard solution to make injections (at least in duplicate) into the HPLC system varying the injected volume.
5. Recommended mass amounts on column are 40 to 400 ng (10–100 µL).
6. Make two to four injections of each volume.
7. Perform regression analysis on the data, plotting peak area units versus µg.
8. Using the Mes-IMPY external standard, calculate the slope of calibration line (**M**) while forcing the Y-axis intercept of calibration line through zero (i.e. **b = 0**). Print out the curve along with the line equation and correlation coefficient ($\mathbf{r^2}$) of the curve and place curve in Document 9 section of CMC.
9. The equation for calculating the mass amount of Mes-IMPY, **Y**, in micrograms is: **Y = Mx** where x = peak area via computer integration.

[^{11}C]MeS-IMPY FOR INJECTION: STANDARD OPERATING PROCEDURES

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SOP # QA308

Acceptance Testing of Desmethyl MeS-IMPY and Reference Compound

Approved by: _____Initials_____ Date: _____

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

Purpose: to validate chemical purity and chemical identity of desmethyl MeS-IMPY and MeS-IMPY for production of [^{11}C]MeS-IMPY for Injection

1. Overview

The radiopharmaceutical production of [^{11}C]MeS-IMPY for Injection for the National Institute of Mental Health requires the use of desmethyl MeS-IMPY (methyl 3-(2-(4-(dimethylamino)phenyl)imidazo[1,2-*a*]pyridin-6-ylthio)propanoate) as a precursor for C-11 methylation. The standard operating procedure (SOP) for acceptance testing of desmethyl MeS-IMPY is described below. Acceptance testing is required to validate chemical purity and chemical identity of desmethyl MeS-IMPY. Upon acceptance of desmethyl MeS-IMPY, the desmethyl MeS-IMPY may be released for the production of [^{11}C]MeS-IMPY. Upon release, the desmethyl MeS-IMPY is to be tested annually thereafter for chemical purity (via HPLC, section 2.2) and chemical identity (via LC-MS, section 2.3).

This SOP also contains testing methods and acceptance criteria for a standard sample of MeS-IMPY (section 3 of SOP #QA308). This non-radioactive material is utilized in the quality control testing of [^{11}C]MeS-IMPY for Injection for the determination of specific radioactivity and chemical identity.

2. Acceptance testing of Desmethyl MeS-IMPY

2.1 Chemical identity via Proton NMR

Weigh out ca. 2.0 mg of desmethyl MeS-IMPY in a clean HPLC autosampler vial. Record the weight data in Document 9 of CMC section. Add ca. 0.8 mL of DMSO (Aldrich, Inc. cat no. 444766-1PAK, 99.96 %) to dissolve the desmethyl MeS-IMPY. Transfer this solution to a clean NMR tube. Acquire high field (≥ 300 MHz) proton NMR spectrum of sample. For sample to

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pass acceptance criterion, characteristic proton resonances listed in Document 9 of CMC must be observed. Record resonances data in the Precursor Compound Acceptance Form in Document 9 of CMC section. Attach NMR spectrum to Document 9 of CMC section.

If characteristic resonances are not observed, the material may not be released for production of [^{11}C]MeS-IMPY for Injection.

2.2 Purity via HPLC

Weigh out approximately 1 mg of DESMETHYL MeS-IMPY in a clean 20 mL screw cap vial. Record the weight in Document 9 of CMC section. Add ca. 10 mL of HPLC grade methanol. Inject 50 μL of solution onto an analytical HPLC system consisting of the following.

Column: Phenomenex Luna (C18; 10 micron, 100A, 4.6 mm x 250 mm; part # 00G-4094-E0) Eluent:isocratic: 25% acetonitrile/ 75 % 0.1 % phosphoric acid. Flow rate: 3.0 mL/min UV Detector: 350 nm

The UV chromatogram is acquired for 15 min. The major peak, corresponding to Desmethyl MeSIMPY, should be observed with a t_R of 6.1 min $\pm$ 1.0 min and it should comprise equal or greater than 95 % of the total peak area between 2.0 and 20 min. Record the result in Document 9 of CMC section whether the material meets these two acceptance criteria. Attach UV chromatogram to Document 9 of CMC section. The chemical purity of the precursor shall be equal or greater than 95 % as determined by HPLC. If the chemical purity is less than 95 %, desmethyl MeS-IMPY may be re-purified by reverse phase HPLC or column chromatography and resubmitted for acceptance testing.

2.3 Chemical Identity via LC-MS

Dilute 100 μL of the desmethyl MeS-IMPY solution (prepared in section 2.2) with 900 μL of 0.5 % acetic acid in water. Inject 10 μL of this solution into Thermo-Finnigan's LC-Q Deca LC-MS system. Acquisition parameters are as follows: (a) Sheath Gas flow Rate (Arb) = 45, Spray Voltage (kV) = 4.50, Capillary Temp (ºC) = 260.00, Capillary Voltage (V) = 40.00 (b) Start delay = 2.0 min, acquire time = 11.00 min, scan type = full scan, Mass Range : 185.00 – 500.00, Polaity : positive ion mode

LC Column: Phenomenex C18, 5 micron, 2.0 mm (ID) x 150 mm, part number: 00F-4252-80, s/no: 202276-6.

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LC Solvent gradient program:

Solvent A = 90 % water, 10 % methanol, 0.5 % acetic acid. B = methanol 0.5 % acetic acid

Time in minute	Solvent A	Solvent B	Flow (µL/min)
0.00	70	30	150
6.00	20	80	150
12.00	20	80	150
13.00	70	30	250

Identify desmethyl MeS-IMPY by detecting protonated parent ion (M+1) 356.1 at ca. 6.4 min. Fill out the table LC/MS in Precursor Compound Acceptance Form in Document 9 of CMC. Attach total ion chromatogram to Document 9 of CMC section.

The criteria outlined in section 2 above are required and sufficient for each new batch of desmethyl MeS-IMPY. The desmethyl MeS-IMPY has an initial expiration period of one year from the date of acceptance.

3. Acceptance testing of MeS-IMPY

3.1 Chemical identity via Proton NMR

Weigh out ca. 2.0 mg MeS-IMPY in a clean HPLC autosampler vial. Record the weight in Document 9 of CMC. Add ca. 0.8 mL of CD_3OD (Aldrich, Inc. cat no. 444758-1PAK, 99.96 %) to dissolve the MeS-IMPY. Transfer this solution to a clean NMR tube. Acquire high field (≥ 300 MHz) proton NMR spectrum of sample. For sample to pass acceptance criterion the characteristic proton resonances listed in Reference Compound Acceptance Form in Document 9 of CMC must be observed. Record resonances in the reference compound acceptance testing Forms in Document 9 of CMC section. Attach NMR spectrum to Document 9 of CMC section.

If characteristic resonances are not observed, the material may not be released for determination of chemical identity and specific activity of [^{11}C]MeS-IMPY for Injection.

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3.2 Purity via HPLC

Weigh out approximately 1.0 mg of MeS-IMPY in a clean 20 mL screw cap vial. Record the weight in Document 9 of CMC section. Add 10 mL of methanol. Inject 50 μL of solution onto an analytical HPLC system consisting of the following:

Column: Phenomenex Luna (C18; 10 micron, 100A, 4.6 mm x 250 mm; part # 00G-4094-E0) Eluent:isocratic: 25% acetonitrile/ 75 % 0.1 % phosphoric acid. Flow rate: 3.0 mL/min UV Detector: 350 nm

The UV chromatogram is acquired for 15 min. The major peak, corresponding to MeS-IMPY, should be observed with a t_R of 4.1 min ± 1.0 min and it should comprise equal or greater than 95 % of the total peak area between 2.0 and 20 min. Record the result in Document 9 of CMC acceptance testing form whether the material meets these two acceptance criteria. Attach UV chromatogram to Document 9 of CMC section. The chemical purity of the MeS-IMPY standard shall be equal or greater than 95 % as determined by HPLC. If the chemical purity is less than 95 %, MeSIMPY may be repurified by reverse phase HPLC or column chromatography and resubmitted for acceptance testing.

3.3 Chemical identity via LC-MS

Dilute 100 μL of the MeS-IMPY solution (prepared in section 3.2) with 900 μL of 0.5 % acetic acid in water. Inject 10 μl of this solution into Thermo-Finnigan's LC-Q Deca LC-MS system. The same acquisition parameters and LC Method used for desmethyl MeS-IMPY are employed (see 2.3). The MeS-IMPY peak is observed at ca. 5.1 min on the total ion chromatogram. Identify MeS-IMPY by detecting protonated parent ion (M+1) 284.1. Fill out the table in Document 9 acceptance testing forms of CMC section. Attach total ion chromatogram to Document 9 of CMC section. The criteria outlined in section 3 above are required and sufficient for each new batch of MeS-IMPY. The MeS-IMPY has an initial expiration period of one year from the date of acceptance.

4. Archiving of Precursor and Reference compound-Acceptance Testing and Forms

Acceptance Testing and Forms obtained as described in SOP # QA308 shall be stored in Document 9 of CMC section of IND application. Annually thereafter, the precursor and reference compound shall be tested according to this SOP (# QA308). The newly generated

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document entitled, “Precursor and Reference compound-Acceptance Testing and Forms” shall be stored in Document 9 of CMC section of IND application along with the expired test forms.

Attachment

This attachment lists the materials and equipments, which are used in the SOPs on Page 1.

Materials:

Item	Name	Name of supplier	Quality grade and catalog #
1	0.22 μm Sterile 4 mm vent filter; Millex GV	Millipore	SLGV0004SL
2	0.22 μm Sterile 25 mm Syringe Driven Filter Unit; Millex MP	Millipore	SLMP L25 SS
3	10 mL Sterile serum vial	Abbott Laboratories	5816-11
4	10 mL sodium chloride injection	American Pharmaceutical Partners, Inc.	USP; NDC 63323-186-10
5	0.5 cc sterile insulin syringe	Becton-Dickinson	329465
6	Polyethylene/polypropylene sterile syringe, 1 cc	Aldrich	Z23,072-3
7	Polyethylene/polypropylene sterile syringe, 3 cc	Aldrich	Z11,685-8
8	Polyethylene/polypropylene sterile syringe, 10 cc	Aldrich	Z11,687-4
9	Polyethylene/polypropylene sterile syringe, 20 cc	Aldrich	Z11,688-2
10	Sterile, 60 mL disposable, luer lock syringe	Aldrich	Z19,231-7
11	Microliter syringe, 5 μL	Vici	160021
12	Microliter syringe, 25 μL	Alltech	84880
13	Microliter syringe, 100 μL	Hamilton	84886
14	Microliter syringe, 250 μL	Hamilton	84889
15	Glass syringe, 5 mL	Hamilton	81520
16	1 L graduated cylinder	VWR	24711-182
17	Magnetic stir bar	VWR	58948-988
18	Magnetic stir bar retriever	VWR	58949-287

19	Spatula	NIH stock room	
20	Acetone	Fisher Scientific	A929-4
21	Hydrogen	Matheson	Research Purity; G2146101
22	Helium	Roberts Oxygen	Research Purity; R104 A3
23	Nitrogen	Roberts Oxygen	Ultra High Purity; MS121371018 for Bioscan
24	Water	EM Science	HPLC grade; EMWX0004-1
25	Phosphoric acid, 85%	EM Science	1.00563
26	Pressure regulator to test filter integrity	Porter Instrument Co.	40000AMVS60
27	Vacuum pump	Welch	model # 8917A, s/n: EF041372
28	Swiss boy remote lift jack/rotary evaporator	Heidolph	511-10000-03-1
29	Sterile 20 G 1.5” needle	Becton-Dickinson	W12552
30	Sterile 21 G 2” needle	Becton-Dickinson	W12562
31	Semi prep column; C-18 Luna 10 micron, 10 mm × 250 mm	Phenomenex	00G-4094-N0
32	Analytical column; C-18 Luna, 10 micron, 4.6 mm × 250 mm	Phenomenex	00G-4094-E0
33	Back pressure regulator	Upchurch Scientific, Inc.	P-787
34	1/16 inch (O.D.) Teflon tubing	Valco Instruments	TTF-120-25
35	1/8 inch (O.D.) Teflon tubing	Valco	TTF-260-25

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		Instruments	
36	Iodine	Mallinckrodt	0984
37	Quartz column	Quartz Scientific	390T008D500
38	Ascarite	Thomas Scientific	C049U90
39	Quartz wool	Alltech	4033
40	Anti-Static Airjet	GC Electronics	19-8495-SF
41	0.45 micron nylon membrane filter	Phenomenex	AF0-0504
42	10 g weight standard	Thomas	7021-3w
43	1.0 and 10.0 mg weight standards	ICL calibration labs	2817
44	Methanol, HPLC grade	Burdick and Jackson	230-4
45	Acetonitrile HPLC grade	Burdick and Jackson	017-4
46	Acetonitrile	Aldrich	271004-12×100ML
47	*tert*-Butylimino-tris(dimethylamino) phosphorane	Fluka	445363-5ML
48	Sodium bicarbonate, 8.4%	Abott Laboratories	S6297-250G
49	Isopropanol, HPLC grade	Alfa Aesar	22906
50	Ethanol, USP	Warner-Graham	64-17-5
51	25 mL glass vial	Alltech	98868
52	1 mL V-vial	Alltech	98226
53	Alcohol Prep	Kendall	6818
54	Mes-IMPY	This Laboratory	
55	Desmethyl Mes-IMPY	This Laboratory	
56	Polysorbate 80, N. F.	J. T. Baker	4117
57	DMSO	Aldrich	276855-12×100ML

Materials, equipment and reagents can be substituted with equivalent materials, equipment and reagents. All substitutions must be approved by Change Control Committee and change documented.

[^{11}C]MeS-IMPY FOR INJECTION: STANDARD OPERATING PROCEDURES

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Instruments and equipment:

Operation/Function	Manufacturer	Model	Serial #
Radiosynthesis	General Electric	GE PETtrace Methyl Iodide Micro Lab	27740
Radiosynthesis	Bioscan	Autoloop system	TW24312483
HPLC purification	Beckman Coulter	System Gold 126 model	3522367
HPLC analytical QC	Beckman Coulter	System Gold 118 model	342-1037
UV absorbance detection in HPLC purification	Beckman Coulter	System Gold 166 detector	312-2185
Radioactivity detection in HPLC purification	Bioscan	Flow-Count PIN detector	G00363-02
Radioactivity measurement	Biodex	AtomLab300 dose calibrator HC#4	01332706
Mass measurement/volume of [^{11}C]Mes-IMPY for Injection	acculab	PP-250B	492AN025
Mass measurement of standard Mes-IMPY and Desmethyl Mes-IMPY	Sartorius	CP225D	13907271