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

PET Radiopharmaceutical Sciences Section,
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SOP # GP101

[^{11}C]*dLop for Injection*: Preparation of HPLC Mobile Phases

Approved by: V. W. Pike Initials: VWP Date: 10/16/07

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

Purpose: To prepare mobile phase required for the HPLC Quality Control analysis of [^{11}C]*dLop for Injection*:

Procedure:

1. Preparation of 1 L preparative HPLC mobile phase (2mM ammonium hydroxide (NH_4OH) in water). Quantities may be scaled as needed.
   1.1. To 1 L HPLC grade water, add 2 ± 0.1 mL 2M NH_4OH to the water (a 1 mL sterile syringe may be used).
   1.2. Mix thoroughly
   1.3. Vacuum filter the mobile phase through a 0.45 µm nylon filter.
   1.4. 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 one week 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 (40% acetonitrile: 60% 0.1% TFA in water). Quantities may be scaled as needed.
   2.1. In a 1-L graduated cylinder, add 0.60 L HPLC grade water.
   2.2. Fill to the 1 L mark with HPLC grade acetonitrile.
   2.3. Add 600 ± 10 µL TFA (a 1 mL sterile syringe may be used).
   2.4. Mix thoroughly
   2.5. Vacuum filter through a 0.45 µm nylon filter.
   2.6. 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 one week 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 a 10 µm inlet filter.

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SOP # GP102

Cleaning Procedures for Radiosynthesis Apparatus

Approved by: V. W. Pike Initials: VWP Date: 11/16/07

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

Purpose: Cleaning Procedures for the Synthia Radiosynthesis apparatus

1. Pre-synthesis
   1.1. Set up the vials and reagents according to the instructions found in SOP# MP 201, section 1.13, Reagent Setup.
   1.2. Fill the syringe reservoir with HPLC grade water.
   1.3. Run the Synthia recipe **dLop CLEANING**.
   1.4. Transfer Lines
      1.4.1. 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.
      1.4.2. 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.
   1.5. Formulation Line
      1.5.1. Push 20 mL (min volume) of USP grade absolute alcohol through the formulation line and dry it by repeatedly flushing air from the syringe through the line.
   1.6. Collection line
      1.6.1. Run 100% acetonitrile through the prep HPLC system. This should be done with only a span of tubing and no column in-line.
      1.6.2. Use manual collection valve V 5 to flush at least 5 mL of acetonitrile through the collection line.
      1.6.3. Disconnect the collection line and manually push through 20 mL (min volume) of USP grade absolute alcohol and reconnect the line
2. Post synthesis
   2.1. Column
      2.1.1. After each synthesis the preparative and analytical columns are washed with 100 % acetonitrile for a minimum of 20 column volumes.
   2.2. Synthia Apparatus (should be performed when residual radioactivity is below detectable levels).

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2.2.1. Fill the syringe reservoir with 70% aqueous ethanol.

2.2.2. Fill F11 and F12 with absolute ethanol and F31 with 70% aqueous ethanol.

2.2.3. Run the Synthia Recipe **dLop CLEANING**

2.2.4. Remove the HPLC column and replace it with a span of tubing. Run 100% acetonitrile and 50% acetonitrile (aq) through pumps B and A respectively.

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SOP # GP103

Cleaning Procedure for Radiochemistry Glassware

Approved by: V. W Pike Initials: VWP Date: 10/16/07

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

Purpose: Cleaning procedures for production glassware

Procedure

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

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: VWP Date: 10/16/07

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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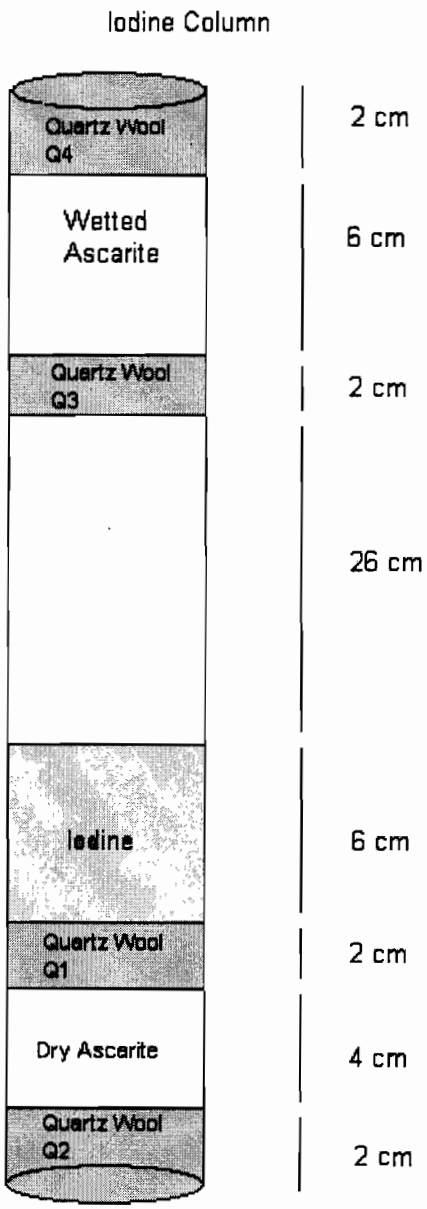
Figure 1: Diagram of packed Iodine Column



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SOP # MP 201

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

Approved by: J. W Pike Initials: VWP Date: 10/16/07

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

1. Setup
   1.1. Ensure the Iodine column has not been used more than eight times.
   1.2. Ensure the power is on to all peripheral devices.
   1.3. Turn target inlet valve ON and request a target flush from a cyclotron engineer, if required.
   1.4. Confirm that the Valco six-way valve is set to position 3
   1.5. Confirm that the three-way valve is set to "Cryotrap" position
   1.6. Run a Prep sequence while monitoring RMA, RMB and RMC
      1.6.1. 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

   1.7. Connect a flow meter to the [^{11}C]Iodomethane trapping station, position 2.
   1.8. Run Leak Check 1 on GE Microlab
      1.8.1. Flip Valve 6 (Hot Cell 4) to **ON** position and measure flow rate on flow meter. Flow rate is typically no less than 15 mL/ min.
      1.8.2. Flip Valve 6 (Hot Cell 4) to **OFF** position and check that the system is leak tight (RMB flow meter typically drops to zero in less than 3 mins).
      1.8.3. Cycle through Leak Checks 2 – 6 to complete the Leak Test.
   1.9. Verify the ionization chamber by measuring the ^{57}Co and ^{137}Cs standards. Compare the measurements to the expected measurements for the day's date. Record the measurements in the Quality Control Record and in the tracking spreadsheet. Report any measurement that is not within ± 5% of the expected measurement.

1.10. Verify the portable balance by measuring the mass of a 10 g NIST traceable calibrated standard weight. The weight should measure 10 ± 0.1g. Record the measurement on the Master Batch Record.

1.11. Install two 2" 21G needles (vent and [^{11}C]Iodomethane transfer needles) to position 2 of the trapping station.

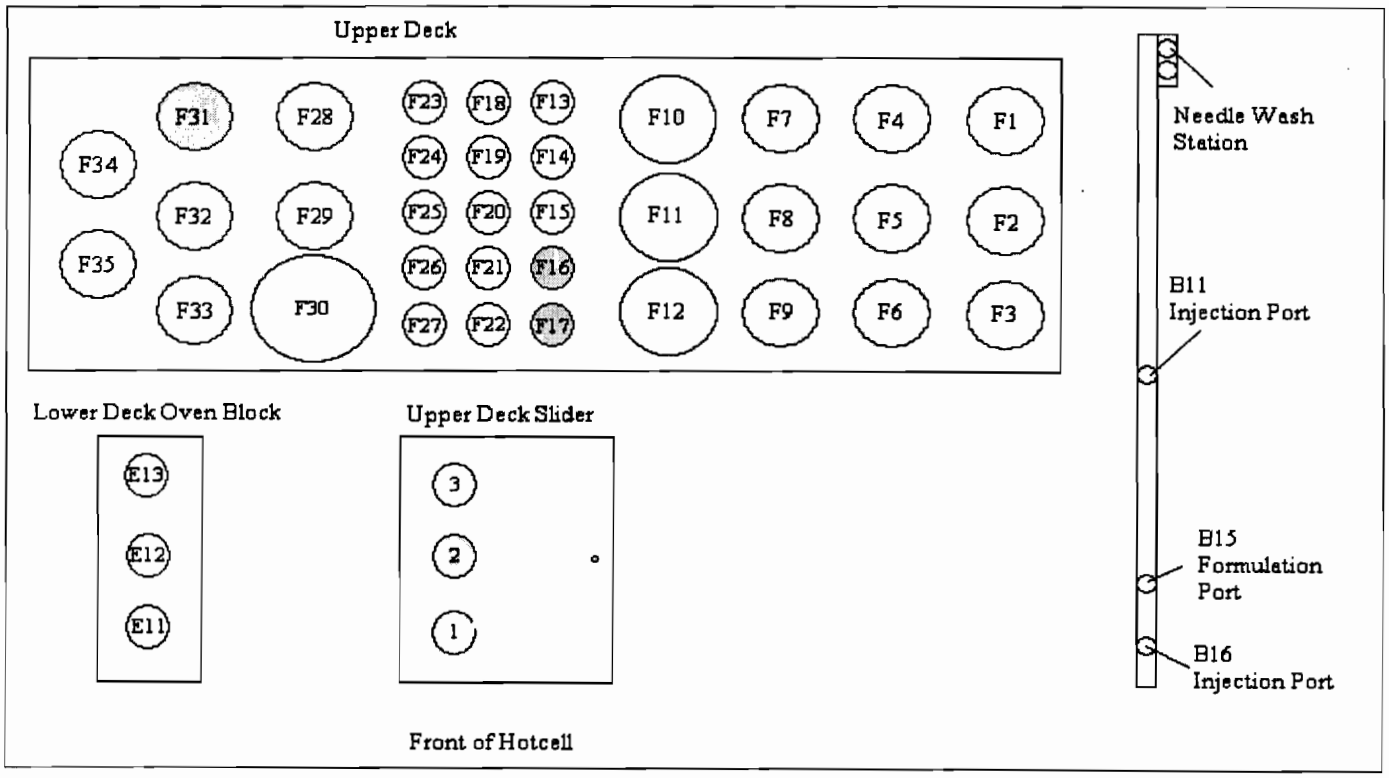
1.12. Cleaning formulation, collection and transfer lines

1.12.1. Clean the formulation, collection and transfer lines according to SOP# GP102, section 1.4, 1.5 and respectively.

1.13. Reagent Setup

1.13.1. Set up all vials required by Table 1 using the Diagram in Figure 2 for guidance.[1]

Figure 2: Diagram of Synthia Deck Layout



[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.

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Table 1

Position	Tube Type	Solvent/ Material	Position	Tube Type	Solvent/ Material
F31	10 mL round bottom	70:30 Absolute Ethanol: HPLC grade Water	F17	1.7 mL Autosampler vial	70:30 Absolute Ethanol: HPLC grade Water
F16	1.7 mL Autosampler vial	70:30 Absolute Ethanol: HPLC grade Water	Slider position 2	Reaction Vial	Precursor, KOH, DMSO

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

1.14.1.1. Weigh 1.0 ± 0.1mg precursor in a tared, high recovery auto-sampler vial and cap the vial.

1.14.1.2. Weigh 5.0 ± 0.1mg of potassium hydroxide in a tared, high recovery auto-sampler vial and cap the vial.

1.15. Waste containers and cold traps

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

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

1.15.3. Add dry ice-ethanol (or other appropriate solvent) slurry to the rotary evaporator condenser and vacuum pump traps.

1.16. Pre-synthesis Cleaning

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

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

1.16.3. Start the recipe.

1.17. Dose vial

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

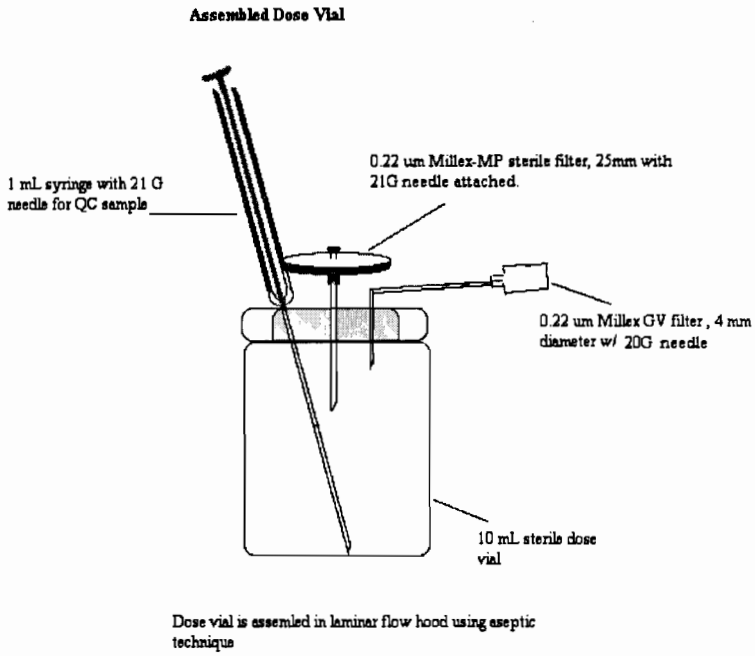
1.17.2. Prepare the laminar flow hood for operations.

1.17.2.1. 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.

Tared 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
QC sampling syringe	

1.17.2.2. 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.

Figure 3



1.17.3. Install the dose vial to the Formulation line once CLEANING is complete.

1.18. HPLC Setup

1.18.1. Preparative

1.18.1.1. 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'.

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

1.18.1.3. Equilibrate the column for a minimum of 10 column volumes at initial conditions (refer to Table 2). The initial pressure for a new column is typically 2500 psi at 6 mL/min when fully equilibrated. The flow may be turned down once the column is equilibrated.

1.18.2. Analytical

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

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

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1.18.2.3. Equilibrate the column for a minimum of 10 column volumes at initial conditions. The initial pressure for a new column is typically 2600 psi at 2.5 mL/min once at full equilibration. The flow may be turned down once the column is equilibrated.

1.18.3. System Suitability for the analytical HPLC

1.18.3.1. Inject dLop standard (100 ng typical). Refer to SOP # QA304, Analytical HPLC Quality Control Method, for acceptance of the standard injection.

1.18.3.2. 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
Detector	Beckman 166	Beckman 166 or 168
Column	Gemini10µm, C18, 150 x 10 mm	Prodigy 10 µm, 150 x 4.6 mm
Flow Rate	6 mL/min	2.5 mL/min
Typical initial pressure	2500 psi	2600 psi
Gradient/ Isocratic	Gradient	Isocratic
Mobile Phase A	2 mM NH_4OH (aq)	0.1 % TFA in 40% MeCN(aq)
Mobile Phase B	Acetonitrile	—
UV Wavelength	225 nm	225 nm
Bioscan Setting	2M	20 K
Method Name	dLop Prep	dLop QC
Trigger	External	External
Sample loop size	5 mL	200 µL

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SOP # MP 202

Production of [^{11}C]dLop for Injection Part 2: Synthesis and Formulation

Approved by: V. W. Pike Initials: VWP Date: 10/16/07

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

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

1. Synthesis

1.1. [^{11}C]Iodomethane Production and Trapping

1.1.1. Once cleaning is complete, close the Synthia program "**dLop CLEANING**".

1.1.2. Restart Visual Chemistry as required. Select 'Run Synthesis' after restart. Select the recipe "**dLop SYNTHESIS**" (refer to Appendix B).

1.1.3. The Synthia recipe will prompt the operator to enter a Reaction Temperature (°C) and Time (seconds).

Reaction Temperature	80 °C
Reaction Time	300 secs

1.1.4. 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

1.1.5. When Step 7 "Methane waste" on the 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 the Synthia program to move down the needles into the reaction vessel.

1.1.6. When the radioactivity is maximized in the reaction vial (about 3–4 min after [^{11}C]methyl iodide release), follow the Synthia instructions to stop gas flow.

1.2. Reaction and Purification

Note: 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. During this time, the preparative HPLC should be made ready for injection (HPLC method = dLop Prep.met) before proceeding as the Synthia recipe loads the injector loop and begins the HPLC run automatically. Ensure that the flow rate is at 6 mL/min. Check that the pressure is within the expected range.

1.2.1. After a brief cool-down, the Synthia recipe will dilute the reaction mixture with 500 µL of water and inject onto the preparative HPLC.

1.2.2. Turn on the vacuum pump and raise the water bath up to the flask shortly before the peak is expected to elute.

1.2.3. Collect the product peak by switching manual control valve V5 **ON**. Refer to Appendix D for a sample preparative trace.

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1.2.4. Switch off manual control valve V5 once collection is complete.

1.2.5. Concentrate the product to dryness under reduced pressure.

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

2. Formulation

2.1. Add saline (10 mL, USP) through the formulation line.

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

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

3. Completion

3.1. Complete the Master Batch Record.

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

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SOP # QA 301

Release of *[^{11}C]dLop for Injection*

Approved by: V. W. Pike Initials: VWP Date: 10/16/07

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Purpose: Description of release procedure and criteria for [^{11}C]dLop for Injection. Clarification of release and post-release test requirements.

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	Procedures	Testing schedule
Appearance	Formulated dose is clear, colorless and particulate free	Visual Inspection	Completed before product release
Bacterial endotoxins (LAL)	Less than 175 EU in injectable volume	LAL test kit procedure	Test completed post-release
Chemical purity	Maximum volume contains NMT 20 µg of carrier and 2.0 µg dLop equivalent impurity	HPLC QC Procedure	Completed before product release
Membrane filter integrity	Sterile 0.22 µm filters are used once. Each membrane tested by bubble point test	Pressure gauge transducer. No bubbles at 45 p.s.i.	Completed before product release
pH	4.0–8.0	pH paper.	Completed before product release
Radiochemical identity	Retention time within ± 1.0 min of a standard injection of dLop	HPLC QC Procedure	Completed before product release
Radiochemical purity	NLT[2] 95 %[^{11}C]dLop	HPLC QC Procedure.	Completed before product release
Radionuclidic identity	The measured half-life is between 20.4 ± 2 min	Calculated half life from two measurements at least 3 minutes apart.	Completed before product release
Residual solvents	≤ 4.1 mg acetonitrile in the injectable dose[3] and ≤ 1 x 10^5 ng/µL ethanol	Gas chromatography with flame ionization detection.	Completed before product release
Specific radioactivity	NLT[2] 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	Test completed post-release

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

2. Labeling and Release

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

[^{11}C]dLop for Injection

Sterile, apyrogenic saline solution for intravenous administration
Caution: New drug limited by Federal law to investigational use only
1 h after calibration Half-life of ^{11}C is 20.4 min
Concentration:_____mCi/mL Vol.:_____mL
Activity:_____mCi Time:_____
Calib. Date:_____ Lot #:_____

2.1. 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 must be completed and acceptance criteria met within the time-frames specified below. Refer to SOP #QA302 for detailed procedures.

Test	Test Description	Acceptance Criteria
Bacterial endotoxins	EndoSafe®-PTS *Limulus* amebocyte lysate (LAL) test	Amount of endotoxin not to exceed 175 EU in injected volume
Sterility	Aerobic and anaerobic bacterial growth test	No growth observed

3.1. Quality control test for bacterial endotoxins and sterility are to be performed on each batch of *[^{11}C]dLop for Injection*. However, because of the time needed to perform these tests, the dose may be released for injection before test completion. Bacterial endotoxin testing should be completed within 24 h of EOS of *[^{11}C]dLop for Injection*.

3.2. 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 or personnel are closed or unavailable but should be completed as soon as possible.

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

4. Batch Record and Data -

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

- Preparative HPLC data attached

Quality Control Record:

- Form contains summary of the pre-release quality control results
- Analytical HPLC Reports attached
- GC report

Post Release Test Record: Endotoxin and Sterility:

- Form contains summary of post-release quality control results (label, pyrogen testing, sterility testing, volatile organics testing)
- Sterility Report
- GC Report attached

Calculations Worksheet

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

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SOP # QA 302

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

Approved by: V. W Pike Initials: VWP Date: 10/16/07

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

Purpose: **Description of all pre- and post-release quality control testing required for *[^{11}C]dLop for Injection*.**

1. **Sampling *[^{11}C]dLop for Injection***
   - 1.1. Use the sterile sampling syringe, previously inserted into the dose vial under aseptic conditions, to remove a 500 µL (minimum) sample.
   - 1.2. Dispense 300 µL (minimum) to a clean vial for HPLC analysis
   - 1.3. Dispense 200 µL (minimum) to a pyrogen-free test tube.
   - 1.4. Measure the activity in the dose vial after the QC sample has been removed. The total activity should be greater than 20 mCi at EOS. The time and activity of this measurement is recorded in the batch record.
2. **Release Tests**

   These tests must be completed prior to release of the *[^{11}C]dLop for Injection* from the production facility and all resultant data recorded on the Quality Control Record.

   2.1. pH

   2.1.1. Dispense 1-3 drops of *[^{11}C]dLop for Injection* on to a strip of narrow-range pH paper. The indicated result should be between 4.0 and 8.0.

   2.2. Membrane Filter Integrity

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

   2.2.2. Submerge the tip of the needle in water contained in a transparent vessel.

   2.2.3. With the compressed air valve closed, attach the air line inside the hot cell to the filter inlet. Ensure the dial is lowered so that the initial pressure will be less than 20 psi and open the air valve.

   2.2.4. Slowly increase the pressure to 45 psi and observe the needle outlet. If no bubbles are observed at 45 psi, the filter passes the integrity test.

   2.2.5. Lower the pressure to below 20 psi and close the compressed air valve.

   2.3. Appearance

   2.3.1. Visually inspect the contents of the dose vial. The product should be a clear, colorless liquid free of particulates or cloudiness.

2.4. HPLC Analysis and Resulting Calculations

2.4.1. Using a Hamilton syringe (or equivalent) fitted with a blunt tip for HPLC injection, remove 100 µL *[^{11}C]dLop for Injection* from the quality control vial (Step 1.2.). Measure the syringe in the ionization chamber the time of this measurement is recorded as the End of Synthesis time (EOS).

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

2.4.3. Integrate the UV trace. Integrate the BioScan trace.

2.4.4. Measure the background activity by immersing the empty dipper into the ionization chamber.

2.4.5. 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.

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

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

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

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

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

$$\%Purity = \frac{\text{Product Peak Area}}{\text{Total Peak Area}} \times 100 \quad \textbf{Eq. 3}$$

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

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

2.4.9. Chemical and Radiochemical Identity

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

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2.4.10. 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:

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

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

Specific Activity is calculated by:

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

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

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

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

3. Residual Solvent

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

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

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

3.4. 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.

3.5. 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).

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4. Post-Release Tests

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

4.1. Bacterial Endotoxins

Note: Steps 4.1.1.-4.1.3. may be performed before the [11C]dLop for Injection *synthesis is complete. The test should be initiated as soon as possible following the synthesis, but the dose may be released for injection before test is completed.*

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

4.1.2. Remove a test cartridge from storage and allow to come to room temperature. If the box is new, the certificate of analysis should be filed in the EndoSafe binder.

4.1.3. 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 (yymmdd).
PROMPT: **Sample ID**	Enter the sample ID (dLop)
PROMPT: **Dilution**	Enter the dilution factor (20)
PROMPT: **Add sample and press enter**	Load sample as follows when ready.

4.1.4. Transfer 50 µL of *[11C]dLop for Injection* from the pyrogen-free test tube 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.

4.1.5. When the test is complete (approximately 15 minutes) 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.

4.1.6. Record the test results on the Post-Release Test 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 Post-Release Test Record.

4.2. 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]dLop for 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.*

4.2.1. Confirm the absence of radioactivity in the sample using a pancake GM detector.

4.2.2. Prepare the laminar flow hood for aseptic use.

4.2.3. 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.**

4.2.4. 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.

4.2.5. 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.

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4.2.6. Add approximately 100 µL to each of the Bactec vials. 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 submitted form with the Batch Record until results are returned in approximately 10 – 14 business days.

4.2.7. When results are returned, indicate result on Post-Release Test Record and file with Batch Record.

4.2.8. *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.

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SOP # QA 303

Analysis of Organic Residues by Gas Chromatography

Approved by: V. W. Pike Initials: VWP Date: 10/16/07

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

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

1. **Operation**
   1.1. Verify that the instrument is ready for operations.
   1.2. Verify that the water level in the H_2-90 hydrogen generator is sufficient.
   1.3. Verify that the H_2 pressure is at approximately 28 psi and that the He tank pressure is about 60 psi
   1.4. Verify that the vials in solvent positions A and B have sufficient DI water.
   1.5. Verify that FID is lit and the background signal is about 5.
   1.6. Load the method 'ISPRCN.M'
   1.7. Add equal amounts of *[^{11}C]dLop for Injection* (test sample) and the calibrated propionitrile standard (50 mL of each is typical).into a prepared auto sampler with insert if required. Make sure that no air bubbles are present.
   1.8. Load the prepared sample into position. Start the run. The run may be stopped and analysis performed at 3.5 minutes.

   The sample is acceptable if the amount of acetonitrile (MeCN, t_R *ca.* 2.67 min) is less than 4.1×10^2 ng and the amount of ethanol (EtOH, t_R *ca.* 2.27 min) is less than 1×10^5 ng/µL based on a 1 µL injection.

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

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Table 1. Materials and equipment

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

Table 2. Method (ISPRCN.M) parameters.

Injection port: split sample injection split ratio of 20: 1 T = 250 °C	**Carrier gas:** Helium 2 mL/min
Column temperature gradient: Time / Temperature / Duration 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	**Detector:** FID 250 °C H_2 40 mL/min and air at 450 mL/min. He make-up 45 mL/min. Detector
Autosampler: Syringe size 10 µL Sample injection volume 1 µL	**Needle/Syringe wash:** Before injection 4 After injection 2

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SOP # QA 304

Analytical HPLC Quality Control Method

Approved by: V. W. Pike Initials: VWP Date: 10/16/07

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

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

1. Preparation

Prepare the HPLC system according to the parameters listed in the table below.

Component/Parameter	Requirement/Setting
HPLC Pump	Beckman 126
HPLC Detector	Beckman 166 or 168
Column	Phenomenex Prodigy 10 µm, 4.6 x 150mm
Flow rate	2.5 mL/min
Typical pump pressure	2.5 – 2.8 kpsi
Mobile phase	40 : 60 acetonitrile:0.1% trifluoroacetic acid (aq)
Isocratic/gradient	Isocratic
UV wavelength	225 nm
Bioscan setting	20 K for 50-2000 µCi injection, may be adjusted
Instrument trigger	External
Sample loop size	100 or 200 µL

1.1. Equilibrate the column at initial conditions for a minimum of 20 column volumes. The initial pressure is typically 2.5-2.8 kpsi at 2.5 mL/min once fully equilibrated. The flow 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 dLop reference standard (typically 100 ng). After the dLop has eluted (approximately 7.0 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 calibration curve should be made.

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

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3. Quality control analysis of *[^{11}C]dLop for Injection*.
   3.1. Prepare and measure a sample for injection according to the procedures described in SOP # QA 302, Section 1: Sampling *[^{11}C]dLop for Injection*
      3.1.1. Start a single run and inject the sample. Enter the pre-injection and background radioactivity measurements in the calculations worksheet.[4]
      3.1.2. After the run is complete (typically 7 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.
      3.1.3. Enter the carrier peak area and the sum of the areas of any impurity peaks in the calculations worksheet. Some peaks present in the trace may be attributed to the sterile filter or the ascorbic acid present in the formulation vehicle and therefore may be ignored in the purity analysis. See formulation vehicle blank HPLC chromatogram in Appendix D for representative chromatogram.

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

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SOP # QA 305

Preparation of dLop Standard Solution and HPLC Calibration Curve

Approved by: V W Pike Initials: VWP Date: 10/16/07

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

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

1. Preparation of a 100ng/µL Stock solution of Reference FBR
   - 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 dLop reference standard into the flask. Record the exact mass on Form #QA305.
   - 1.3. Dilute to the 25-mL mark with anhydrous dimethyl sulfoxide. Mix thoroughly.
   - 1.4. Transfer the contents of the flask to a septum-sealed 30 mL sterile dose vial. Label the vial with "dLop Stock", calculated concentration and date of preparation. Record calculated concentration on Form #QA305.
   - 1.5. Store the vial in a - 20 °C freezer when not in use.

2. Prepare a 1 ng/µL (approximate) solution of reference dLop for HPLC injection.
   - 2.1. Transfer precisely 100 µL of the dLop stock solution to a clean 10-mL volumetric flask.
   - 2.2. Dilute to the 10-mL mark with water. Mix thoroughly.
   - 2.3. Transfer the contents of the flask to a septum-sealed 10-mL sterile dose vial. Label the vial with "dLop for HPLC", calculated concentration and date of preparation. Record the calculated concentration on Form #QA305.
   - 2.4. Store the vial in a - 20 °C freezer 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]dLop for Injection.*
   - 3.1. Remove the "dLop for HPLC" standard 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]dLop for Injection*, approximately 25 - 200 ng ($5 \times 10^{-5} - 4 \times 10^{-4}$ µmol). 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 dLop is 463.01 g/mol.

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, replicates for the point 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 in the *[^{11}C]dLop for Injection* Supplementary Records Binder, stored with the *[^{11}C]dLop for Injection* batch records.

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SOP # QA306

dLop Precursor and Reference Acceptance Criteria

Approved by: V. W. Pike Initials: VWP Date: 10/16/07

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

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

1. Overview

The procedure at PRSS/ MIB/ NIMH for the radiosynthesis of *[11C]dLop* requires *4-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylbutanamide* (hereafter referred to as dLop precursor).

Reference dLop, *4-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-N-methyl-2,2-diphenylbutanamide* 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 *[11C]dLop* for Injection.

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

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

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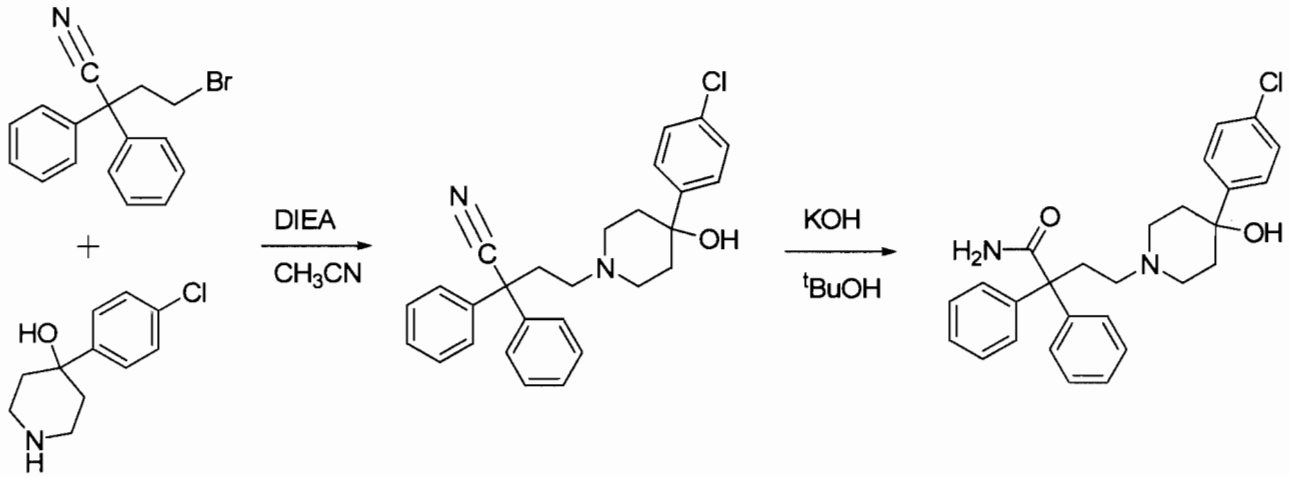
2. Synthetic Schemes for Preparation of Precursor and Reference Standards

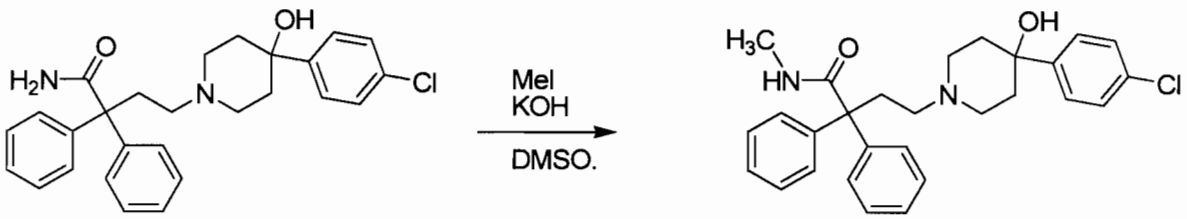
2.1. The precursor compound, *4-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylbutanamide,* is synthesized from commercially available 4-(4-Chlorophenyl)-4-hydroxypiperidin and 4-Bromo-2,2-diphenylbutyronitrile in two steps, according to Scheme A.

Scheme A. dLop Precursor



2.2. The non-radioactive dLop reference compound is prepared by N-methylation of the amide substituent using methyl iodide, as seen in Scheme B.

Scheme B. dLop Reference Compound



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3. Acceptance Test Method Parameters/ Sample Preparation

3.1. Material Source

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

3.2. Acceptance Tests[5]

3.2.1.NMR

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

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

Methods

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

3.2.2.LC-MS

Sample Prep
20 µg/mL dLop precursor in 50% MeOH (aq).
20 µg/ mL dLop reference material in 50% MeOH (aq).

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

Method Parameters

	dLop reference	**dLop precursor**
Column:	Luna 3 µm, 50 x 2 mm	Luna 5 µm, 150 x 2 mm
Flow Rate:	150 µL/ min	150 µL/ min
Gradient/ Isocratic	Isocratic	Gradient
Mobile Phase:	Methanol: water: acetic acid	Methanol: water: acetic acid
	10: 90: 0.5 %v/v	10: 90: 0.5 %v/v
Injection Volume:	1 µL	1 µL
Ionization:	Electrospray	Electrospray
Detection Range:	*m/z* 150 to 750	*m/z* 150 to 750

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

3.2.3. HPLC

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

Instrument
Beckman Coulter 126 pump and 166 or 168 detector with 32 Karat, ver. 7 software. Phenomenex Prodigy 10 µm, 250 x 4.6 mm column.

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

3.3. Acceptance Criteria

^{1}H-NMR and ^{13}C-NMR

Consistent with the structure. Refer to reference spectra attached

LC-MS

Molecular ion consistent with structure. Refer to reference spectra attached.

HPLC

Purity at 225 nm greater than 95%. Refer to reference spectra attached.

4. Documentation

4.1. The Precursor and Reference Acceptance Form should be filled out completely with the appropriate spectra attached. The completed form and attachments should be stored in the *[^{11}C]dLop for Injection* Supplementary Records Binder and stored with *[^{11}C]dLop for 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]dLop for 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	017-4
Trifluoroacetic acid	Sigma-Aldrich	299537
Analytical HPLC column (Prodigy 10 µm, 4.6 x 250 mm)	Phenomenex	00G-4244-E0
Semi-prep HPLC column (Gemini C18 5 µm, 10 x 250 mm)	Phenomenex	00G-4435-N0
Dimethyl sulfoxide	Sigma-Aldrich	276855
Potassium Hydroxide	Sigma-Aldrich	
Potassium Hydroxide	Sigma-Aldrich	221473
dLop precursor	PET Radiopharmaceutical Sciences, MIB, NIMH, NIH	AKT-01-017
dLop reference standard	PET Radiopharmaceutical Sciences, MIB, NIMH, NIH OR GlaxoSmithKline, Five Moore Drive, PO Box 13398, Research Triangle, NC, 27709-3398	AKT-01-041 040222/GW827631X
Ethanol, absolute	Warner-Graham	6505001050000
0.9% Sodium chloride for injection, USP	Abraxis Pharmaceutical Products	918610
Ascorbic acid	Sigma-Aldrich	A2218
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	General Electric	Synthia	Cecellia - 001
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 and portable balances	ICL Calibration Labs	ASTM Class 1.0 and 10.0 mg weights	2817
Measurement of HPLC sample for quality control	Hamilton	Microliter syringe, 100 µL	84886
Bacterial endotoxin test	Charles River Laboratories	EndoSafe - PTS	1247 or 1248

Appendix B: Synthia Cecilia Recipes

dLop CLEANING

Row_nr	Object	Function	Arg_1	Arg_2	Arg_3	Arg_4	Label	Comments
1	Dout	Reset_All	#	#	#	#	#	#
2	#	Delay	5	#	#	#	#	Wait 2 s (for system to start)
3	Dilutor	Init	L	N	#	#	#	Initiating the dilutor
4	#	Delay	5	#	#	#	#	Wait 2 s (for system to start)
6	BCD	SET_Position	1	9	#	#	#	
7	BCD	SET_Position	3	1	#	#	#	
29	Robot	Switch_Valve	A	L	#	#	#	Analytical injection valve set on load
30	#	Attention	Clean Analytical Injection port with 2 ml of Ethanol AND HIT OK	#	#	#	#	
31	Robot	Switch_Valve	A	I	#	#	#	Analytical injection valve set on inject
100	#	Print	Initiating cleaning...	#	#	#	#	
300	Robot	Move_XYZ	A1	#	#	#	#	Position rinsing needle
400	Dilutor	Init	L	N	#	#	#	Initiating the dilutor
500	Robot	Switch_Valve	P	I	#	#	#	Activate Mel reactor 1
600	Robot	Switch_Valve	A	I	#	#	#	Activate Mel reactor 1
600	#	SetVariableName	ReactionTemperature	#	#	#	#	
900	Dout	Off	34	#	#	#	#	Sterile needles go up
910	Robot	Switch_Valve	A	L	#	#	#	Analytical injection on load
920	#	Attention	Place 70/30 ethanol/water in vial F31. Hit Okay.	#	#	#	#	

930	Robot	Switch_Valve	A	I	#	#	#	prep injection valve set on Inject
950	Robot	Move_XYZ0	F31	#	#	#	#	
955	Dilutor	Aspirate	L	300	50	N	#	Rinsing the needle
957	Robot	Move_XYZ	F31	#	#	#	#	
959	Dilutor	Aspirate	L	3000	50	N	#	pulling up 3 ml of 70/30 EtOH/water
961	Robot	Move_XYZ	B11	#	#	#	#	
963	Robot	Switch_Valve	P	L	#	#	#	Activate Mel reactor 1
965	Dilutor	Dispense	L	3000	50	N	#	pulling up 3 ml of 70/30 EtOH/water
967	Dilutor	Aspirate	L	5000	50	R	#	pulling up 3 ml of 70/30 EtOH/water
969	Dilutor	Dispense	L	5000	50	N	#	pulling up 3 ml of 70/30 EtOH/water
971	Robot	Switch_Valve	P	I	#	#	#	Activate Mel reactor 1
1000	#	Delay	1	#	#	#	#	Wait 2 s
1200	Dout	Off	36	#	#	#	#	Closing the trapdoor
1350	Dout	On	35	#	#	#	#	Sterile vial slider out from sterile station
1400	Dout	On	4	#	#	#	#	Condition trap tubings
1500	Flow	Set_Flow	1	20	200	#	#	Setting nitrogen flow to 20 ml/min
1600	Dout	Rotor_Valve	6	#	#	#	#	Bypass Mel reactors
3600	Robot	Home	#	#	#	#	#	
10800	#	Attention	Load the sterile DOSE vial.	#	#	#	#	
11200	#	Print	-	#	#	#	#	

11450	#	Attention	Load 70/30 Ethanol/water at positions F17 and F16	#	#	#	#	
70600	Vacuum	Stop	#	#	#	#	#	Turn off vacuum pump
70700	Robot	Home	#	#	#	#	#	Position home
70800	#	Attention	Cleaning is completed.	#	#	#	#	

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dLop Synthesis

Row_nr	Object	Function	Arg_1	Arg_2	Arg_3	Arg_4	Label	Comments
1	Dout	Reset_All	#	#	#	#	#	#
5	#	Delay	5	#	#	#	#	Wait 2 s (for system to start)
9	Robot	Switch_Valve	A	L	#	#	#	Analytical injection valve set on load
10	#	Attention	Clean Analytical Injection port with 2 ml of Ethanol	#	#	#	#	
11	Robot	Switch_Valve	A	I	#	#	#	Analytical injection valve set on inject
15	BCD	SET_Position	1	9	#	#	#	
20	Dilutor	Init	L	N	#	#	#	Initiating the dilutor
30	#	Delay	5	#	#	#	#	Wait 2 s (for system to start)
100	#	Print	Initiating C-11 synthesis via MeI from GE	#	#	#	#	
300	Robot	Move_XYZ	A1	#	#	#	#	Position rinsing needle
400	Dilutor	Init	L	N	#	#	#	Initiating the dilutor
500	Robot	Switch_Valve	P	I	#	#	#	Activate MeI reactor 1
600	#	Set Variable Name	ReactionTemperature	#	#	#	#	
600	Robot	Switch_Valve	A	I	#	#	#	Activate MeI reactor 1
610	#	UserInput	Set the reaction temperature.	ReactionTemperature	#	#	#	
620	Oven	Set_Temperature	6	Reaction Temperature	#	#	#	
630	#	SetVariableName	Rxntime	#	#	#	#	
640	#	UserInput	Set reaction time in seconds.	Rxntime	#	#	#	
650	#	Print	-	#	#	#	#	

660	#	Delay	3	#	#	#	#	
800	Oven	Set_Temperature	8	80	#	#	#	Set evaporator temperature
900	Dout	Off	34	#	#	#	#	Sterile needles go up
1660	BCD	SET_Position	3	1	#	#	#	
1700	#	Attention	Check that all reagents are in position! Remove slider so it doesn't overheat	#	#	#	#	Wait 2 s
1800	#	Ask	Would you like to prime the dilutor?	No_Prime	#	#	#	
1900	#	Print	Priming the dilutor.	#	#	#	#	
2000	Dilutor	Init	L	N	#	#	#	Initiating the dilutor
2100	Dilutor	Aspirate	L	5000	50	R	#	Priming the dilutor / Rinse needle
2200	Dilutor	Dispense	L	5000	20	N	#	Priming the dilutor / Rinse needle
2300	Robot	Move_XYZ	A1	#	#	#	#	Position rinsing needle
2400	Dilutor	Aspirate	L	10000	50	R	#	Priming the dilutor / Rinse needle
2500	Dilutor	Dispense	L	10000	20	N	#	Priming the dilutor / Rinse needle
2600	Dilutor	Aspirate	L	2000	50	R	No_Prime	Priming the dilutor / Rinse needle
2700	Dilutor	Dispense	L	2000	20	N	#	Priming the dilutor / Rinse needle
2800	Robot	Move_XYZ	A2	#	#	#	#	Position rinsing the top of the needle
2900	Dilutor	Aspirate	L	1000	50	R	#	Priming the dilutor / Rinse needle
3000	Dilutor	Dispense	L	1000	10	N	#	Priming the dilutor / Rinse needle
3500	#	Print	-	#	#	#	#	

3600	Robot	Home	#	#	#	#	#	
13400	#	Print	Positioning the reaction slider.	#	#	#	#	
13500	Robot	Move_XYZ	I1	#	#	#	#	Positioning the reaction slider
13550	Dout	Off	33	#	#	#	No_trap_ready	Trap needles go up (if trap station was not ready for use
13600	Robot	Move_XY	I2	800	#	#	#	Positioning the reaction slider
13800	Robot	Move_XYZ	I3	#	#	#	#	Positioning the reaction slider
13900	Robot	Move_XY	I4	800	#	#	#	Positioning the reaction slider
14000	Robot	Move_XYZ0	F10	#	#	#	#	Position over washing solution reservoir
14100	#	Print	-	#	#	#	#	
14300	#	Attention	Make sure Ge Mel output is hooked into position 2 of synthia. Place the precursor v-vial in slider position 2, and press Ok!	#	#	#	#	
14700	Robot	Move_XYZ	I1	#	#	#	J_1	Positioning the reaction slider
14800	Robot	Move_XY	I2	800	#	#	#	Positioning the reaction slider
14900	#	Delay	1	#	#	#	#	Wait 1 s
14950	Dout	Off	4	#	#	#	#	N2 gas flow not to trap station
15000	Dout	On	33	#	#	#	#	Trap needles go down
15100	Robot	Move_XYZ0	F10	#	#	#	#	Removing aspec arm
15200	#	Ask	Is the trap station ready for use?	No_trap_ready	#	#	#	
16600	#	Print	Ready for synthesis/EOB	#	#	#	#	
35300	Robot	Home	#	#	#	#	#	
35350	#	Attention	Press OK when C-11 Mel begins to release	#	#	#	#	

35400	Dout	On	70	#	#	#	#	Trap needles go down
35600	#	Attention	Press OK when C-11 MeI maximizes in precursor solution	#	#	#	#	
35650	Dout	On	26	#	#	#	#	Trap needles go down
35660	Dout	Off	70	#	#	#	#	Trap needles go up
36400	#	Print	Moving reaction vial to the oven.	#	#	#	#	
36500	Robot	Move_XYZ	I1	#	#	#	#	Positioning the reaction slider
36600	Dout	Off	33	#	#	#	#	Trap needles go up
36700	Robot	Move_XY	I2	800	#	#	#	Positioning the reaction slider
36800	Robot	Move_XYZ	I3	#	#	#	#	Positioning the reaction slider
36900	Robot	Move_XY	I4	800	#	#	#	Transferring the reaction vial to the oven
36910	Robot	Move_XY	I5	20	#	#	#	
36920	Robot	Move_R	30	20	Y	-	#	
36930	Robot	Move_R	60	20	Y	+	#	
36940	Robot	Move_R	30	20	Y	-	#	
37300	Oven	Set_Temperature	1	18	#	#	#	Turn off 1st MeI reactor oven
37400	Oven	Set_Temperature	2	18	#	#	#	Turn off 2nd MeI reactor oven
37500	Oven	Set_Temperature	3	18	#	#	#	Turn off 3rd MeI reactor oven
37600	Oven	Set_Temperature	4	18	#	#	#	Turn off 4th MeI reactor oven
37700	Oven	Set_Temperature	5	18	#	#	#	Turn off 5th MeI reactor oven
37800	Robot	Home	#	#	#	#	#	Position home
37900	#	Print	Heating the reaction mixture	#	#	#	#	
38000	#	Delay	Rxntime	#	#	#	#	Alkylation reaction time (s)
38100	Oven	Set_Temperature	6	18	#	#	#	Turn off reaction oven

38200	#	Print	Diluting the reaction mixture with 500uL water.	#	#	#	#	
38300	Dilutor	Aspirate	L	500	20	R	#	Asp. Dilution volume
38400	Robot	Move_XYZ	E12	#	#	#	#	Position reaction vial in oven
38500	Dilutor	Dispense	L	500	5	N	#	Disp. dilution vol
38600	#	Print	Injecting on the preparative HPLC system.	#	#	#	#	
38700	Robot	Move_XYZ0	E12	#	#	#	#	Position over reaction oven
38800	Dilutor	Aspirate	L	150	3	N	#	Aspirating air plug
38900	Robot	Move_XYZ	E12	#	#	#	#	Position reaction vial in oven
39000	Dilutor	Aspirate	L	950	10	N	#	Asp. reaction mixture + dil. Volumev
39100	Robot	Move_XYZ	B11	#	#	#	#	Position preparative injection port
39300	Robot	Switch_Valve	P	L	#	#	#	Preparative injection valve set on Load
39400	Dilutor	Dispense	L	950	5	N	#	Load reaction mixture + dil. Volume on prep injection valve
39410	Dilutor	Dispense	L	150	5	N	#	Dispensing reaction mixture in preparative injection port
39411	Dilutor	Aspirate	L	300	5	R	#	Aspirating water from reservoir…
39412	Dilutor	Dispense	L	300	5	N	#	Dispensing reaction mixture in preparative injection port
39420	#	Delay	1	#	#	#	#	
39500	Robot	Switch_Valve	P	I	#	#	#	Preparative injection valve set on Inject
39600	#	Print	Starting the preparative HPLC system.	#	#	#	#	

39700	Dout	On	56	#	#	#	#	Signalling to start prep HPLC system
39800	#	Delay	2	#	#	#	#	Wait 2 s (for system to start)
39900	Dout	Off	56	#	#	#	#	Stop signalling to prep HPLC
40000	#	Print	Rinsing the needle with H2O	#	#	#	#	
40100	Robot	Move_XYZ	A1	#	#	#	#	Position rinsing needle
40200	Dilutor	Aspirate	L	2000	50	R	#	Rinsing the needle
40300	Dilutor	Dispense	L	2000	50	N	#	Rinsing the needle
40400	Robot	Move_XYZ	A3	#	#	#	#	Position rinsing the needle on the outside
40500	Dilutor	Aspirate	L	1000	50	R	#	Rinsing the needle
40600	Dilutor	Dispense	L	1000	50	N	#	Rinsing the needle
70800	#	Attention	Click okay to perform QC.	#	#	#	#	
70900	Robot	Switch_Valve	A	L	#	#	#	Analytical injection on load
80000	#	Attention	Inject Ethanol to wash the loop and click Okay.	#	#	#	#	
80100	Robot	Switch_Valve	A	I	#	#	#	prep injection valve set on Inject
80200	#	Attention	Click Okay after the column has been equilibrated.	#	#	#	#	
80300	Robot	Switch_Valve	A	L	#	#	NOT_finished_QC	Analytical injection on load
80400	#	Attention	Inject QC sample and Hit OK to start acquisition.	#	#	#	#	
80500	Robot	Switch_Valve	A	I	#	#	#	prep injection valve set on Inject
80600	#	Print	Starting the analytical HPLC	#	#	#	#	
80700	Dout	On	56	#	#	#	#	Signalling to start prep HPLC system
80800	#	Delay	2	#	#	#	#	Wait 2 s (for system to start)

80900	Dout	Off	56	#	#	#	#	Stop signalling to prep HPLC
81000	#	Delay	180	#	#	#	#	Wait 2 s (for system to start)
81100	#	Ask	Are you finished?	NOT_finished_QC	#	#	#	
81200	Robot	Home	#	#	#	#	#	Position rinsing the needle

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Appendix C: 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.

	Radiopharmacy Dose Sheet for [^{11}C] dLop		
Date (mm/dd/yy):			
Batch # (dLop-yymmdd):			
Primary chemist:			301-451-3917 (lab)
Radioconcentration (mCi/mL) =	=B18*10	=C18	
Specific radioactivity (mCi/μmol) =	=B18/B24	=C18	
dLop Carrier concentration (μg/mL) =	=B25*10		
Total volume of formulated [^{11}C]dLop for Injection (mL) =			
Maximum allowable injection volume (mL) =	=MIN(B29:B30)		
Enter slope of calibration curve, M (X= μmol, Y= UV peak area):	374981264.8111		
Date of calibration curve preparation:	39360		
Enter radioactivity in whole dose vial (mCi):			
Enter radioactivity in 100 μL aliquot (mCi):			**EOS Time**
Enter radioactivity remaining in syringe after injection (mCi):			
Net activity in 100 μL aliquot (mCi) =	=B16-B17		
Enter UV peak area of dLop carrier peak:			
Enter sum of areas of all impurity peaks:			
Percent chemical purity of dLop =	=B19/(B19+B20)*100		
Molecular weight of dLop =	462.21		
μmol of dLop in 100 μL aliquot	=B25/B23		
Mass of dLop in 100 μL aliquot (μg) =	=(B19/B12)*B23		
Mass of dLop equivalent impurity in 100 μL aliquot (μg) =	=(B20/B12)*B23		
Maximum allowable injected carrier mass (μg):	20		
Maximum allowable injected impurity mass (μg):	2		
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 (m			
Time between measurements (min)			
Caluclated half-life (min)	=(LN(2)*B33)/LN(B15/B32)		
Homegeneity Check			
Total measured activity @ EOS (whole dose)			
Total calculated activity @ EOS (syringe)			
Percent difference	=ABS(B38-B37)/B38*100		
90% Expected Area			
Expected Area			
110% Expected Area			

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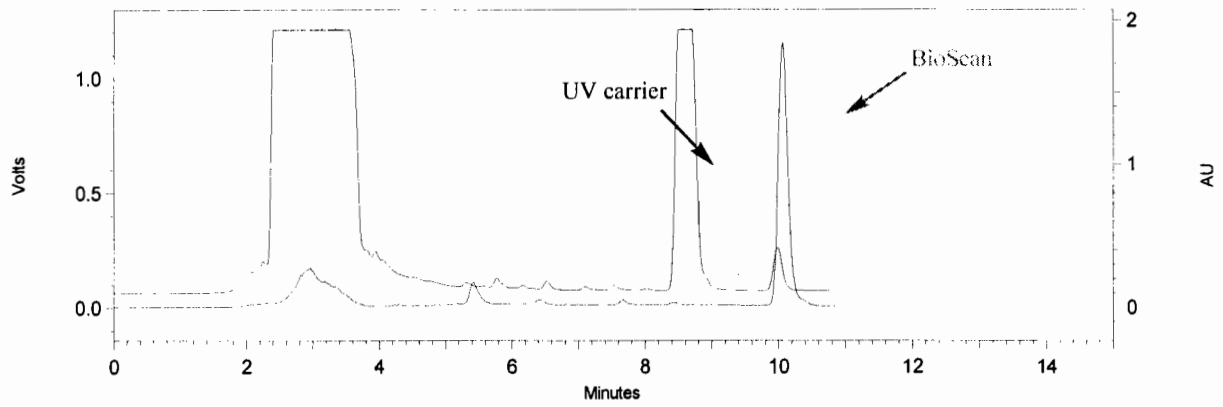
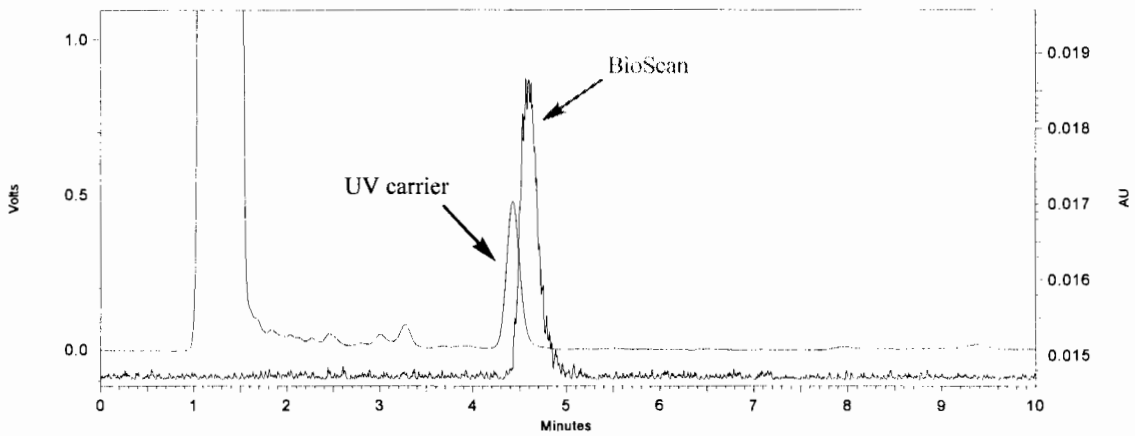
Appendix D: Representative HPLC Chromatograms

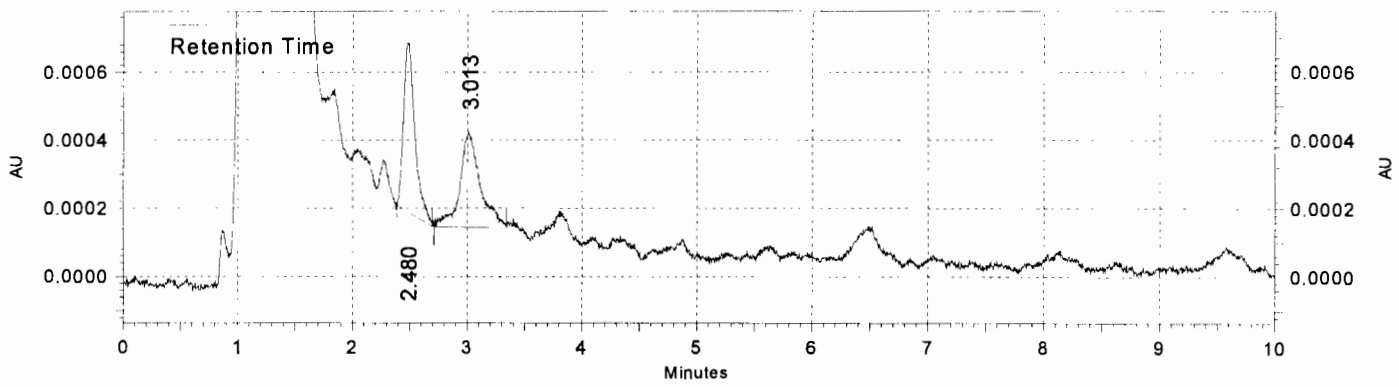
Representative Semi-Preparative HPLC Chromatogram



Representative Analytical HPLC Chromatogram



Representative Analytical HPLC Chromatogram of Formulation Vehicle Blanc



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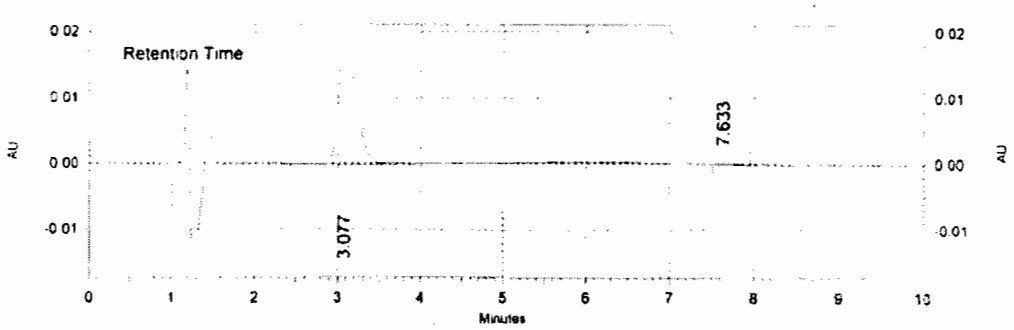
Appendix E: Representative Characterization Data for Precursor and Reference dLop

Precursor Characterization Data, Lot #NL-001-107

dLop QC

Data File:	D:\32Karat\Projects\dLop\Data\dLop Precursor NL-001-107.dat
Method:	D:\32Karat\Projects\DESMETHYL LOPERAMIDE\Method\DLop_analycal.met
Acquired:	10/4/2007 10:19:25 AM
Printed:	10/09/2007 12:36:22 PM
HPLC:	Isocratic 40/60 MeCN/ 0.1 % TFA. (flow rate = 2.5 ml/min, UV at 225 nm. Phenomenex Prodigy 10 micron, 4.6 mm x 250 mm

UV 225



Bioscan Results

Time	Area	Area %	Height	Height %

Det 166 Results

Time	Area	Area %	Height	Height %
3.077	276789	99.33	17334	99.30
7.633	1863	0.67	122	0.70
Totals	278652	100.00	17456	100.00

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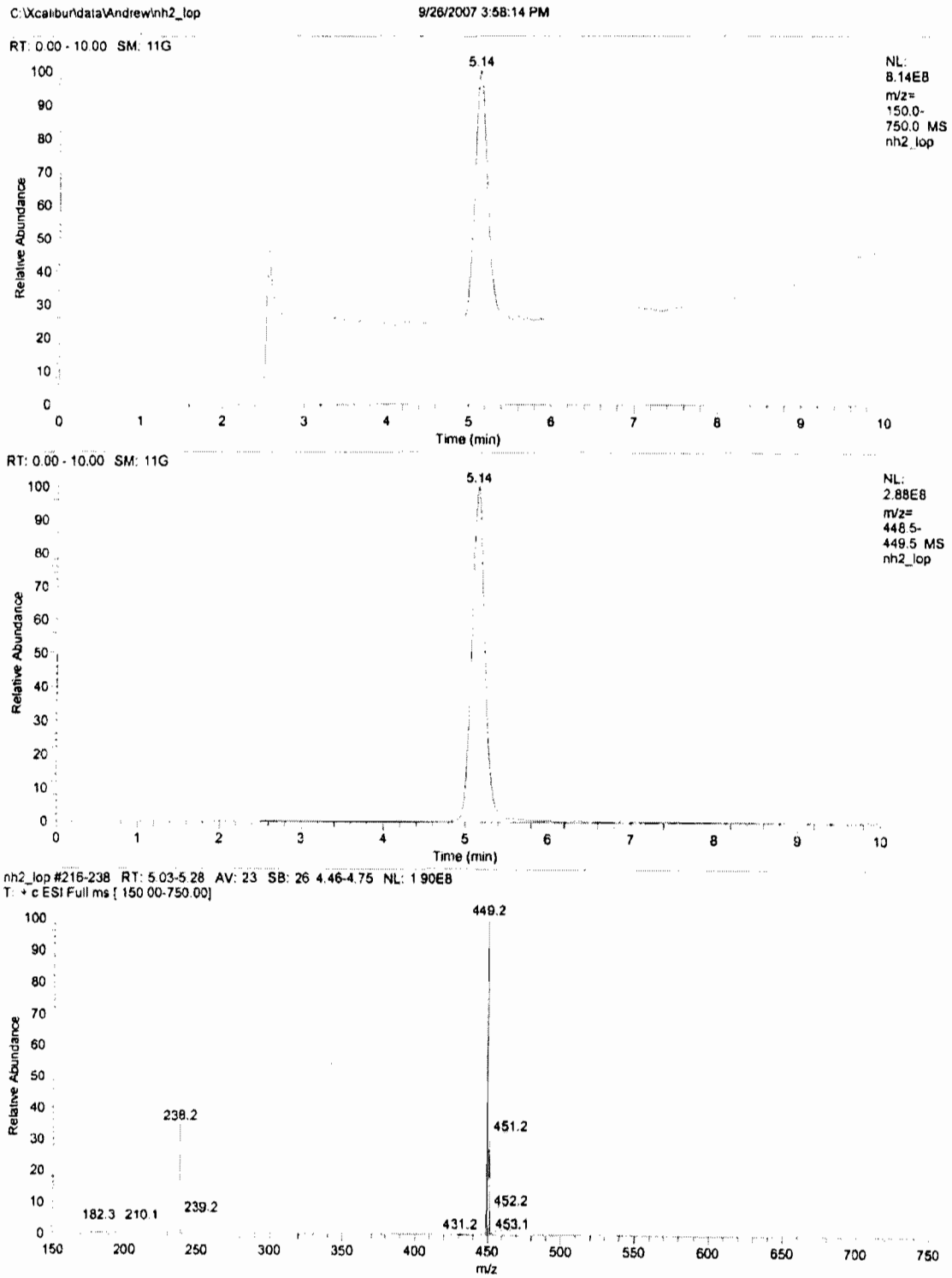
Precursor Characterization Data, Lot # NL-001-107



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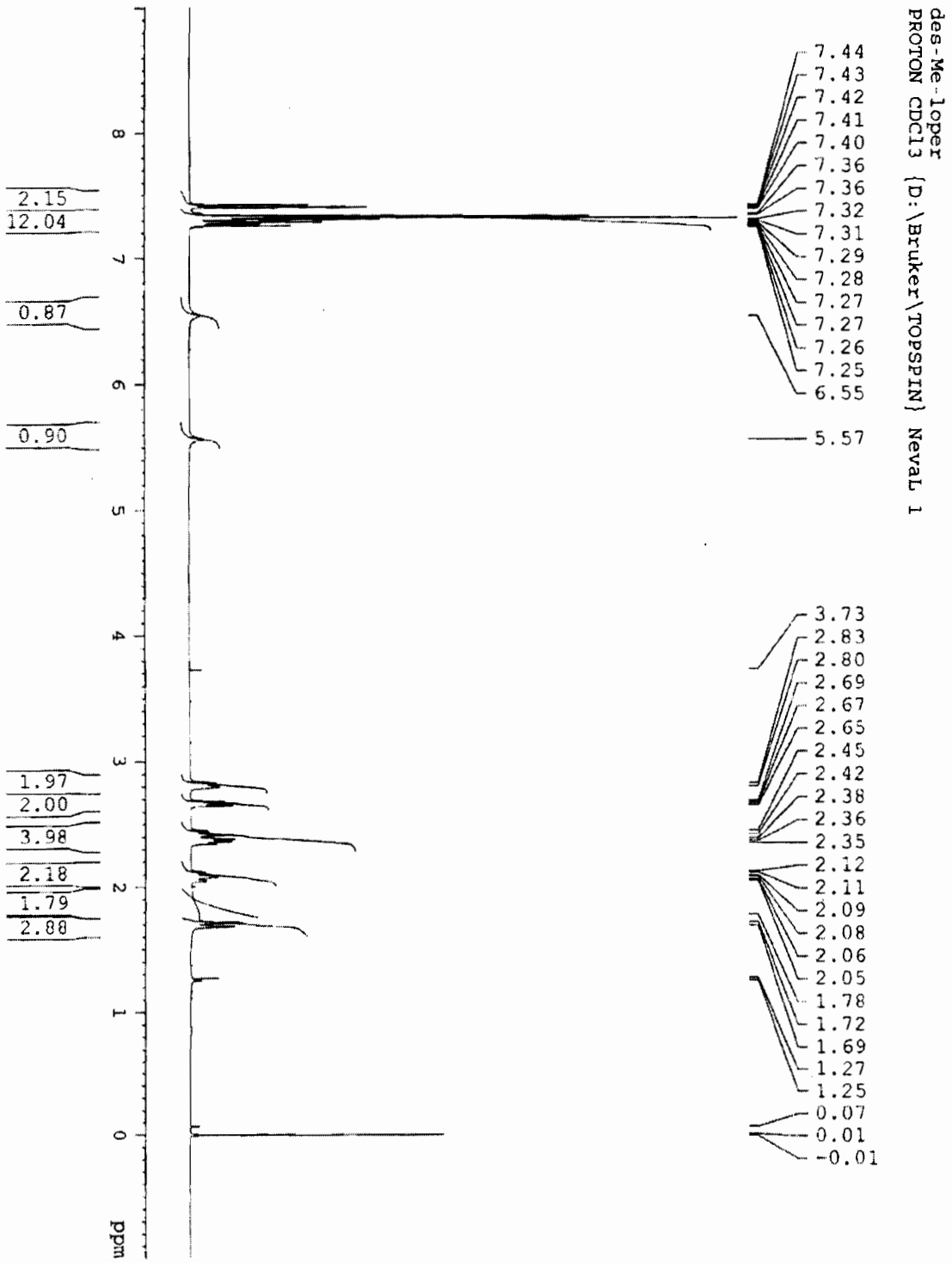
Precursor Characterization Data, Lot # NL-001-107



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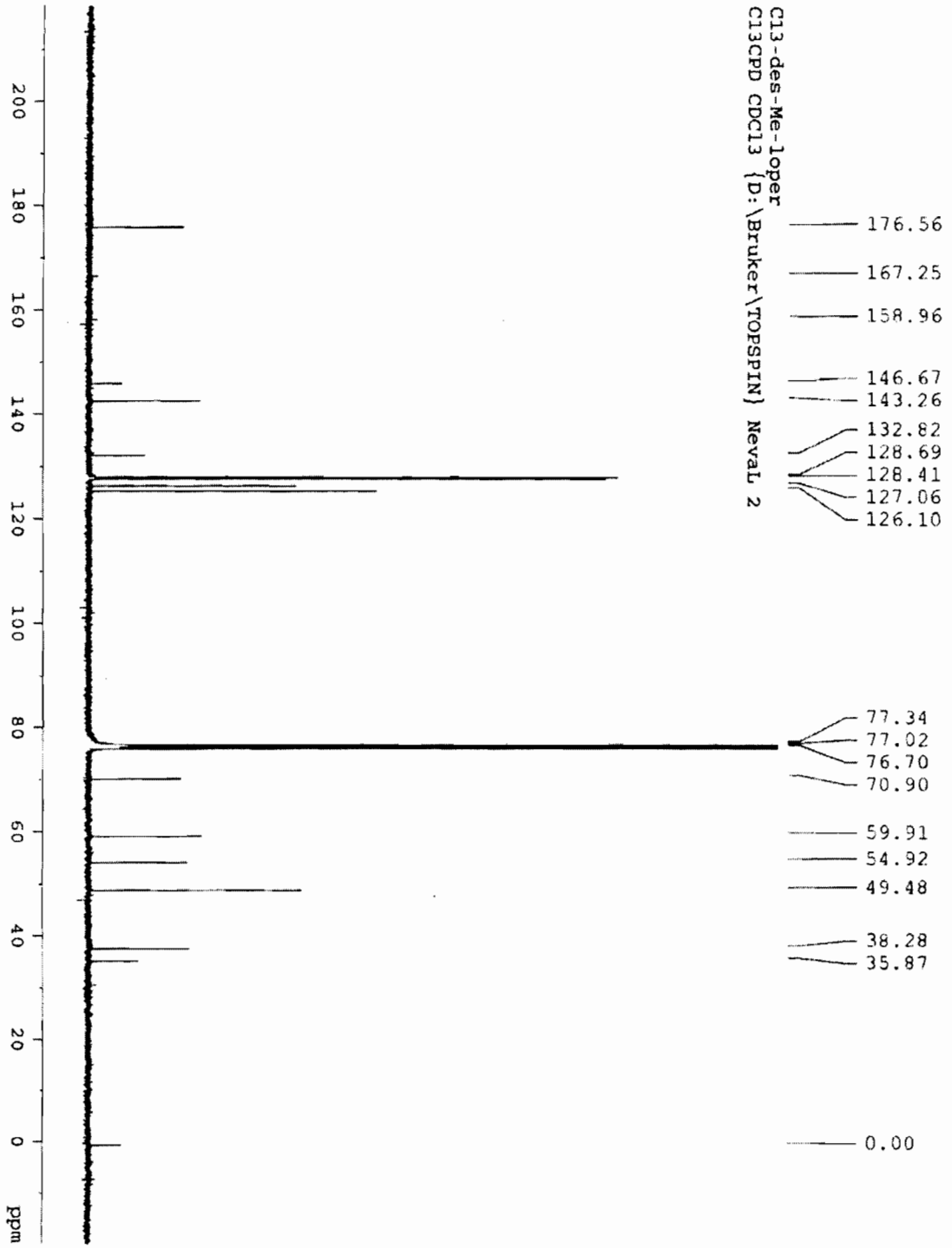
Precursor Characterization Data, Lot # NL-001-107



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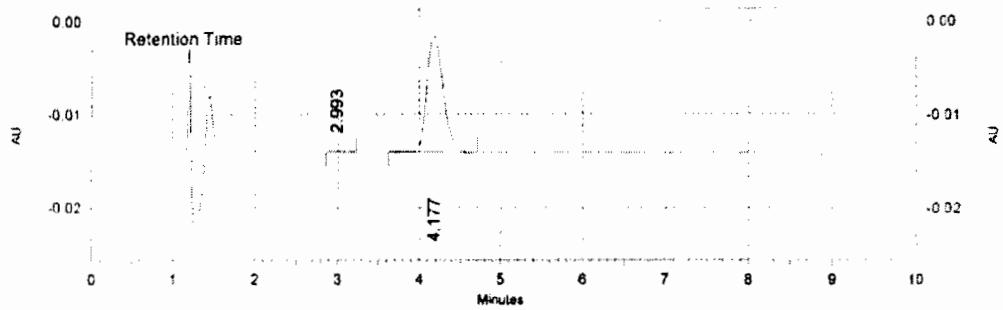
Reference Standard Characterization Data, Lot # 04022

dLop QC

Data File: D:\32Karat\Projects\dLop\Data\dLop Reference GW827631X, Batch# 040222.dat
Method: D:\32Karat\Projects\DESMETHYL LOPERAMIDE\Method\DLop_analycal.met
Acquired: 10/4/2007 12:13:27 PM
Printed: 10/09/2007 01:00:03 PM
HPLC: Isocratic 40/60 MeCN/ 0.1 % TFA. (flow rate = 2.5 ml/min, UV at 225 nm. Phenomenex Prodigy 10 micron, 4.6 mm x 250 mm

UV 225



Bioscan Results

Time	Area	Area %	Height	Height %

Det 166 Results

Time	Area	Area %	Height	Height %
2.993	1452	0.83	130	1.02
4.177	173942	99.17	12584	98.98

Totals	175394	100.00	12714	100.00

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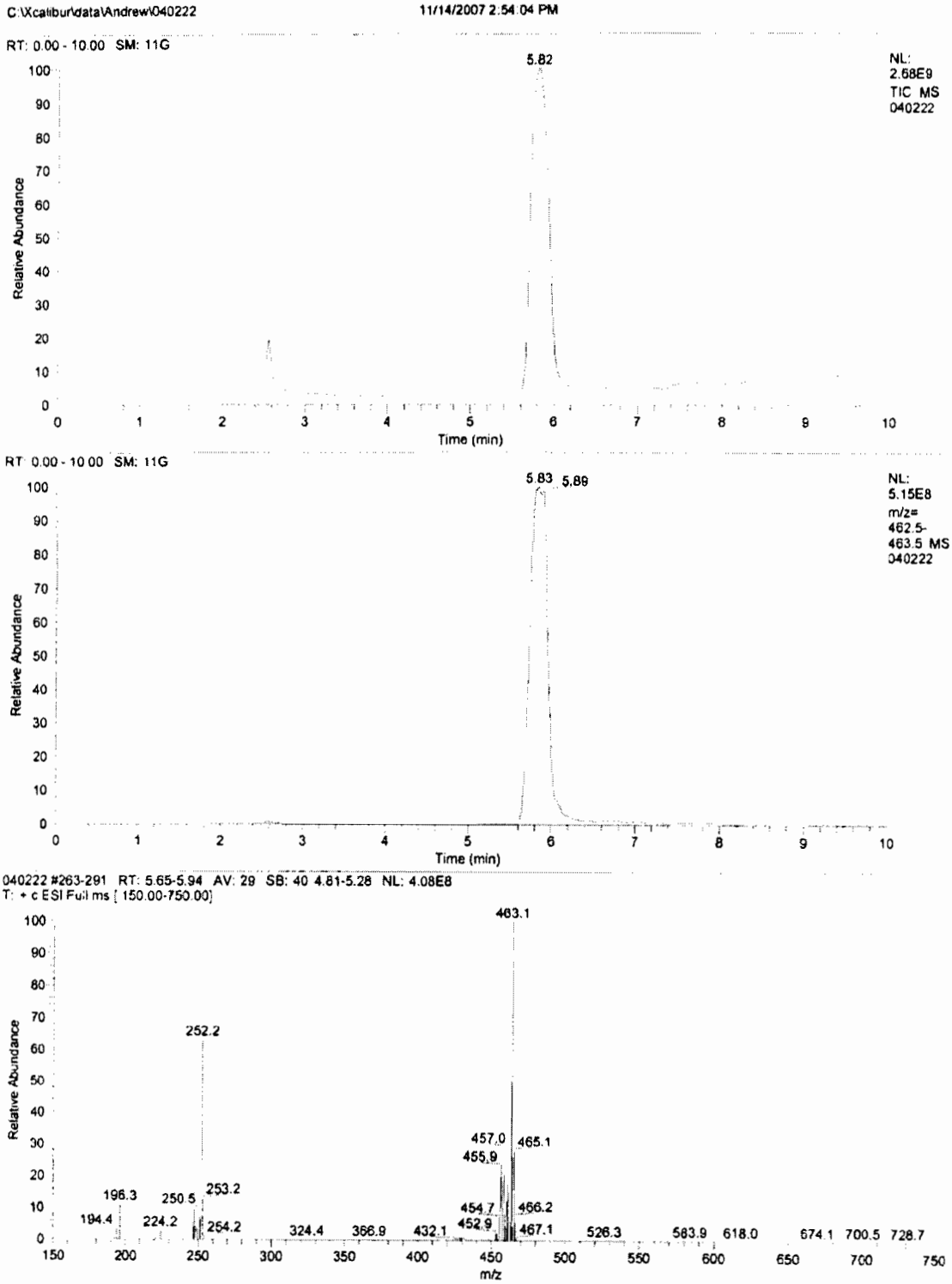
Reference Standard Characterization Data, Lot # 04022



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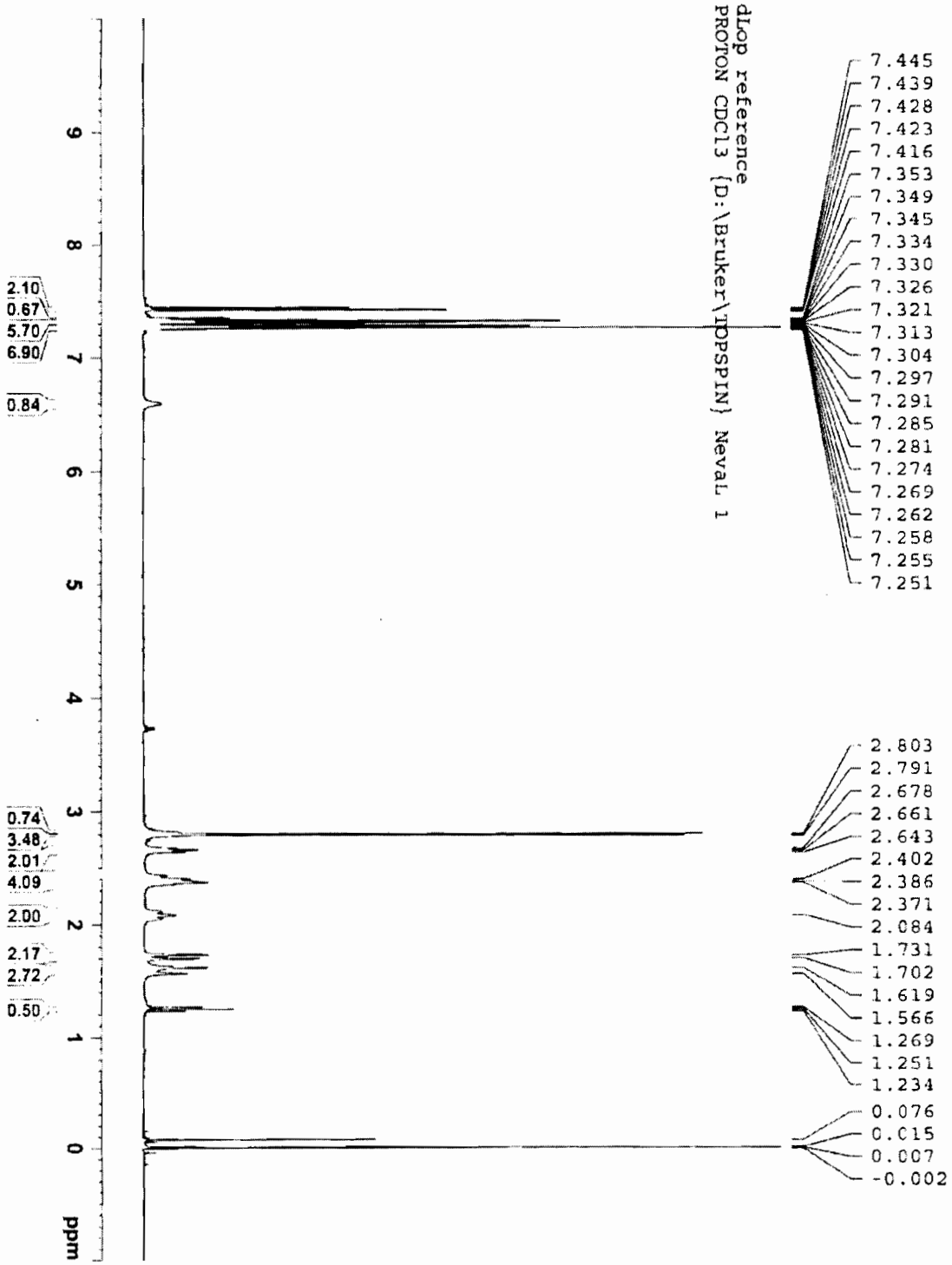
Reference Standard Characterization Data, Lot # 04022



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Reference Standard Characterization Data, Lot # 040222