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Standard Operating Procedure

GEN-Q119

Chemical and Radiochemical Purity by HPLC

Approval Signatures:

Printed Name	Date	Signature	Date
Author:			
Supervisor:			
Regulatory Affairs/QA:			

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Chemical and Radiochemical Purity by HPLC

1. **Purpose:** To describe the quality control procedure for using HPLC to determine the mass concentration and chemical and radiochemical purity of the final 3'-deoxy-3'-[^{18}F]Fluorothymidine ([^{18}F]FLT) product solution.

2. **Scope:** The HPLC method described here will be used for determining the chemical and radiochemical purity of the final product, at the *manufacturing sites* synthesizing [^{18}F]FLT under the *Sponsor's* IND. This quality control procedure is one of the quality control release tests performed on the final product and it must be completed prior to releasing the batch of final product for human administration.

3. **Responsibilities:**
   3.1. *Manufacturing Sites*: To follow this procedure as written and to ensure that all results, control information, and raw data are documented as specified in dedicated Quality Control Laboratory Notebooks and recorded on the appropriate testing forms.

4. **References**
   4.1 USP 26 ‹621› Chromatography
   4.2 USP 26 ‹821› Radioactivity
   4.3 USP 26 ‹823› Radiopharmaceuticals For Positron Emission Tomography—Compounding
   4.4 J.R. Grierson, A.F. Shields, Radiosynthesis of 3'-Deoxy-3'-[^{18}F]fluorothymidine: [^{18}F]FLT for imaging of cellular proliferation *in vivo*, Nucl. Med. Biol. 27 (2000) 143-156.
   4.5 **GEN-Q015**, "Investigating Out of Specification QC Test Results"
   4.6 FDA Draft Guidance PET Drug Products—Current Good Manufacturing Practice (CGMP)—June 2002 (CDER)
   4.7 Machulla H.J. *et al*. Procedure for Routine Synthesis of [^{18}F]FLT in High Activities. *J. Nucl. Med.* **2001**, 42 *(5)*, 257P.
   4.8 Czernecki S. *et al*. Efficient Transformation of Thymidine into 2',3'-Di-dehydro-2',3'-di-deoxy-thymidine (D4T) involving opening of a 2,3'-Anhydro Derivative by Phenylselenol. *Nucleosides & Nucleotides* **1995**, *14*, 1227-1232.
   4.9 Kateman G., Buydens L. Quality Control in Analytical Chemistry, second ed. John Wily & Sons, Inc. New York. 1993. 317 p.
   4.10 **HBS-L**, "Preparation of HPLC Buffer Solution for Purification of [^{18}F]FLT"
   4.11 **HES-L**, "Master Compounding Record for the HPLC Aquasil Column Mobile Phase"
   4.12 **GEN-M120**, "Documentation of Manufacturing Variances"
   4.13 **STDFLT-L**, Master Compounding Record for "Preparation of FLT Standard Solution"

Comment [JL1]: Should put the analytical chem qc ref here.

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5. **Forms**
   - **Form FQC-001**, "[^{18}F]FLT Final Product QC Test Results" Form I
   - **Form IOS-001**, "Investigating Out of Specification (OOS) QC Test Results Form"
   - **Form MVR-001**, "Manufacturing Variance Report Form"
   - **Form Q119**, "Preparation of FLT Standard Solution and Dilutions Form"

6. **Policies**
   N/A

7. **Materials and Equipment**
   7.1. Analytical HPLC Column (Aquasil C_{18} 2.1 mm ID X 150 mm long, 5 µm particles, (Thermo-Hypersil) or equivalent)
   7.2. High Performance Liquid Chromatography (HPLC) System consisting of a high pressure pump, either an auto-sampler/injector, or manual injector; column and flow-through radioactivity detector; & either a variable or multi-wavelength UV detector, with data acquisition system. A mass spectrometer may also be used for the identity test in section 8.3.
   7.3. Non-Radioactive FLT Standard, (**SS-FLT**)
   7.4. **HBS-L**, Preparative Mobile Phase Consisting of 92% 0.01 M Phosphate: 8% Ethanol (v:v)
   7.5. **HES-L**, Analytical Mobile Phase Consisting of 86% HPLC Grade Water: 14% HPLC Grade Methanol (v:v)
   7.6. Automatic pipettors with disposable tips and glass graduated cylinders of appropriate sizes
   7.7. Glass volumetric flasks or glass vials for dilutions

8. **Procedures**
   8.1. Preparation of Standards
   8.1.1. **A Stock FLT standard solutions** consisting of 3'-deoxy-3'-fluorothymidine:e, FLT at 1 mg/mL concentration should be made as directed in compounding record **STDFLT-L**.
   8.1.2. **Dilutions of the stock FLT standard solution:** The stock standard solution should be diluted through several 2-fold to 10-fold dilutions to obtain standard concentrations appropriate for the analysis. Any dilutions made of the stock standard solution should be made using the preparative mobile phase. Use **Form Q119**, "Preparation of FLT Standard Solution and Dilutions Form," to prepare the standard concentration appropriate for analysis.

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8.2. Calibration Curves

8.2.1. Inject a minimum of either five dilutions or five volumes of the standards so that the concentrations measured in the standard curve either bracket the solution to be tested or are all greater than the sample to be tested. The lowest standard to be tested must be less than the maximum allowed, 6.1 µg for the total injected dose corrected for the volume injected. For example, if the injected dose is 10 mL then the minimum acceptable limit, for a 10 mL injection is 6.1µg/10 mL = 0.61 µg/mL. The lowest concentration standard should be < 0.61 µg/mL and the concentration (UV absorbance peak area) of the highest standard must be greater than that for the product sample injection.

Comment [JL2]: We need to determine this amount, I think this minimum value should be 10 ug/mL.or less.

8.2.2. The retention time for FLT is approximately 8.5 minutes. The FLT precursor does not elute from the column with this analysis method. Sample chromatograms are shown in Figures 1-3. All standards must be baseline resolved (resolution > 1.5) for a valid analysis.

Parameters	
UV Detector:	**Set to 254 or 266 nm**
Flow Rate:	**0.3 mL/min**
Eluent:	**14% Methanol: 86% Water (v:v)**
Temperature:	**Room Temperature**
Column:	**Aquasil C_{18}:** 2.1 mm ID X 150 mm long (5 µm particles)

Comment [JL3]: I think this isn't what I am currently using, I need to check this when I get back to the lab.

8.3. Preparation and Analysis of Sample

8.3.1. Inject the sample. (This must be done in triplicate but step 8.3.2 may be done after the first of three sample injections, rinse the injector between injections).

8.3.2. As an identity test, either use a mass spectrometer to determine the mass associated with the [^{18}F]FLT product peak or inject a sample of the [^{18}F]FLT product spiked with sufficient FLT standard to observe the UV absorbance from the standard. Either the mass spectrum must show the mass (M/Z) to be 289 (ES^-) in the mass peak associated with the UV absorbance and radioactivity peaks or the UV absorbance peak from the standard spiked sample must co-elute with the radioactive peak and not show two UV absorbance peaks at or near the radioactivity peak.

8.3.3. The test for release will be the completion of the standard curve and the first product injection meeting criteria for release and the identity test. Two more product injections will follow to assure

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the product FLT chemical analysis obtained from the first injection was correct.

8.4. Data Analysis

8.4.1. Integrate all of the UV responses to obtain the peak areas for the FLT standards, and the UV responses to obtain the peak areas for the FLT and FLT precursor for the product sample.

8.4.2. Determine the equation for the line of regression between the UV responses (area under the curve for the eluted peak) versus concentration.

8.4.3. Determine the correlation coefficient for the linear relationship.

NOTE: If the correlation coefficient is < 0.8, the standards must be rerun and an OOS investigation initiated.

Comment [JL4]: Do we need an out of spec here? I think so.

8.4.4. Do the reverse correlation to determine the concentration of FLT and in the final solution.

8.4.5. Typically the final concentration of the [^{19}F]FLT in the [^{18}F]FLT product to be tested will be between 0.01 and 1 µg/mL. It is possible that the product sample will give a UV absorbance (peak area) that is below or near the limit of quantitation for the UV detector and is much less than the 6.1 µg per sample equivalent signal. If the peak area is less than 10 standard deviations of the baseline peak to peak noise of the UV absorbance signal (this is the limit of quantification), then the product will be reported as not quantifiable with a concentration that is less than the value for the limit of quantitation.

8.4.6. Any other 254 or 266 nm UV absorbing chromatographic peaks that elute after more than 3.0 minutes post injection, ie. post injection and mobile phase disturbances, should be integrated and shown to be less than "12 µg" in the final product. Assume that the molar absorption coefficients for the unknown contaminants are the same as for FLT and that the FLT standard curve is applicable to the unknown contaminants.

8.4.7. Integrate all of the radioactive peaks in the chromatogram. Determine the percent of the total radioactivity that is in the [^{18}F]FLT peak.

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ACCEPTANCE CRITERIA

Radiochemical Purity ≥ 95%
Identity of radioactive peak confirmed by co-injection of FLT or 289 M/Z
Other UV absorbing contaminants < 12 "µg" in the final product
Non Radioactive FLT < 6.1 µg in the final product
Residual Leaving Group (Benzoic Acid) not measured* (*The maximum amount of benzoic acid possible in the final reaction is < 3.5 mg, a nontoxic amount, so benzoic acid is not assayed.)*

8.5.1. Sample chromatograms are presented in Figures 1-3. Figure 1 shows an UV chromatogram of a [^{19}F]FLT standard, the retention time of the ~FLT is 8.5 minutes (k' = 6.1). Figure 2 shows chromatograms from an [^{18}F]FLT product analysis. Figure 3 shows a chromatogram for benzoic acid. The retention time of the benzoate is ~1.7 minutes.

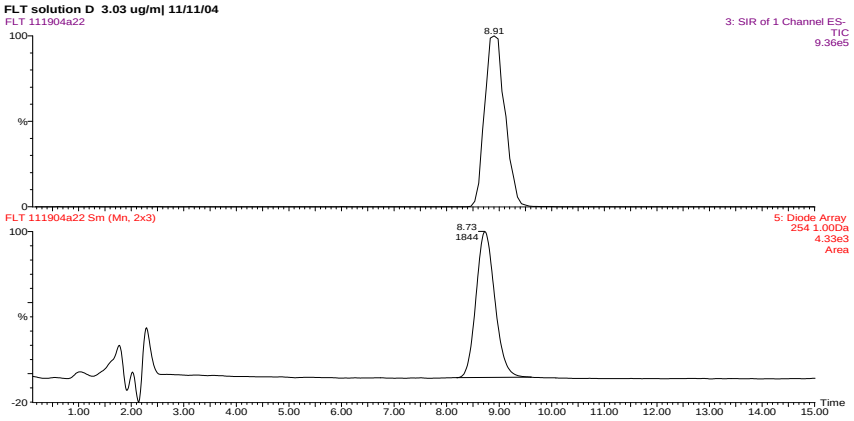

Figure 1: HPLC chromatograms for a 10 µL injection of a 3 µg/mL FLT standard . Bottom trace is UV absorbance at 254 nm. Top trace is of the mass (ES-) of FLT (M/Z 289) confirming the identity of the UV peak. The elution slight time differences are due to the tubing volume between the detectors.

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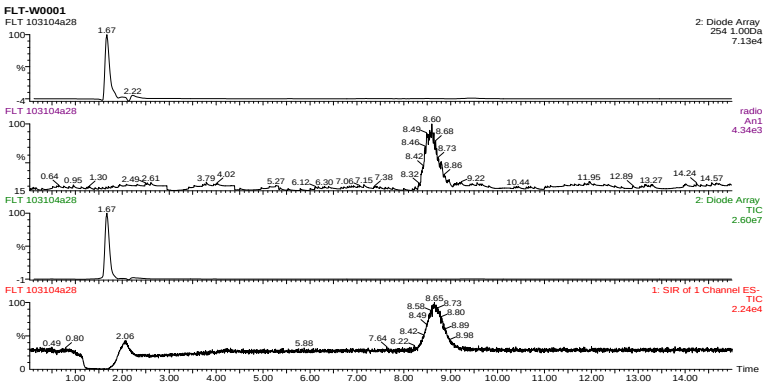

Figure 2: HPLC chromatogram of [^{18}F]FLT product. Bottom trace shows the mass of FLT. Trace second from bottom is UV absorbance from 200 to 400 nm. Trace second from top shows a radioactivity trace and the top trace shows the UV absorbance at 254nm.

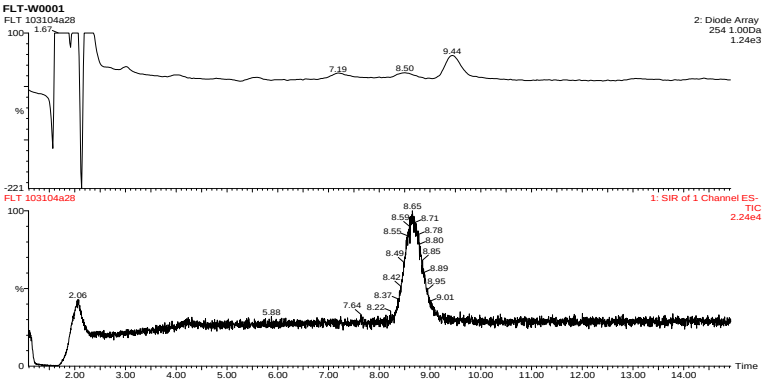

Figure 2b: Enlargement of fig. 2a chromatogram with mass trace (bottom) and UV absorbance (254 nm top) greatly enlarged to show impurities in the final product and the FLT peak. The concentration of [^{19}F]FLT in this product is approximately 0.03 µg/mL.

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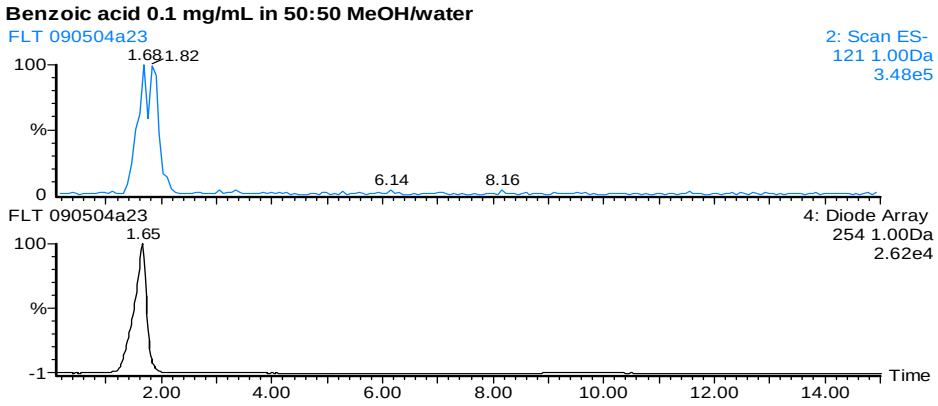

Figure 3 shows a chromatogram of benzoic acid. The bottom trace shows UV absorbance (254nm) and the top trace shows the mass peak (121 negative ion) for benzoic acid.

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REVISION HISTORY

Version Number	Description	Reason for Revision	Date	Author
V0	Original Record	Revised NCI-Q119 Version January 28, 2005 to create this SOP.	March 1, 2005	Teresa R. Zuber

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