Template

"Insert Your Institution Information Here"

Standard Operating Procedure

Leidos-Q324

Chemical and Radiochemical Purity by Analytical HPLC for [^{18}F]DCFBC

1. **Purpose:** To describe the quality control procedure for using HPLC to determine the mass concentration and chemical and radiochemical purity of the final [^{18}F]DCFBC 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 [Place your institution information here] and all contract sites manufacturing [^{18}F]DCFBC under [Place your institution information here] 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:** [Place your institution information here]: 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 28 ‹621› Chromatography
   - 4.2 USP 28 ‹821› Radioactivity
   - 4.3 USP 28 ‹823› Radiopharmaceuticals For Positron Emission Tomography—Compounding

SOP**: Leidos-Q324** Version: **V1**
Effective Date**:____12/11/2013_____** Supersedes**:**

Printed Name	Signature	Date
Author:		
Regulatory Approval:		

Procedure becomes effective on latest date of the two approval signatures above.
Procedure applies to FES IND.

4.4 **NCI-Q015**, "Investigating Out of Specification QC Test Results"
4.5 FDA Draft Guidance PET Drug Products—Current Good Manufacturing Practice (CGMP)—September 2005 (CDER)
4.6 **PHOS30-L,** Master Compounding Record for "Preparation of HPLC Mobile phase for Purification of [^{18}F]DCFBC"
4.7 **ACN50-L,** Master Compounding Record for "Preparation of the [^{18}F]DCFBC HPLC Analytical Mobile Phase"
4.8 **NCI-M120,** "Documentation of Manufacturing Variances"

5. Forms

- **Form FQC-009**, "[^{18}F]DCFBC Final Product QC Test Results" Form
- **Form IOS-001**, "Investigating Out of Specification (OOS) QC Test Results Form"
- **Form MVR-001**, "Manufacturing Variance Report Form"
- **Form Q324**, "Preparation of DCFBC Standard Solution and Dilutions Form"

6. Policies

N/A

7. Materials and Equipment

7.1. Analytical HPLC Column (Luna C_{18}, 5 µm particles, (Phenomenex Luna) or equivalent) (**SS-Luna**)
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 8.3.2.
7.3. Non-Radioactive DCFBC Standard, (**SS-DCFBC**)
7.4. **ACN50-L**, Analytical Mobile Phase Consisting of 50% HPLC grade water: 50% HPLC Grade Acetonitrile:0.1% TFA (v:v:v)
7.5. Automatic pipettors with disposable tips and glass graduated cylinders of appropriate sizes
7.6. Glass volumetric flasks and glass or plastic vials for dilutions

8. Procedures

8.1. Preparation of Standards

8.1.1. **A Stock DCFBC standard solution** consisting of DCFBC at 1.0 mg/mL concentration should be made as directed in **Form Q324**.
8.1.2. **Dilutions of the stock DCFBC standard solution:** Several 10-fold to 2-fold dilutions of the stock standard solution are needed to obtain standard concentrations appropriate for the analysis. All stock standard solution dilutions should be made using the analytical mobile phase (**ACN50-L**). Use **Form Q319**, "Preparation of DCFBC Standard Solution and Dilutions Form," to prepare the standard concentration appropriate for analysis.

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 (**Form Q324**) in the standard curve (Figure 1) 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, 3µg/mL for the injected dose corrected for the volume injected. For example, if the injected dose is 15 mL then the minimum acceptable limit, for a 15 mL injection is 45 µg/15 mL = 3.0 µg/mL. The lowest concentration standard should be < 3.0 µg/mL and the concentration (UV absorbance or Mass peak area) of the highest standard must be greater than that for the product sample injection.

8.2.2. The retention time for DCFBC is approximately 5.5 minutes. The DCFBC precursor does not elute from the column with this analysis method. Sample chromatograms are shown in Figures 2-4. All standards must be baseline resolved for a valid analysis.

Parameters
UV Detector: **Set to 220 nm**
Flow Rate: **0.6 mL/min**
Eluent: **50% ACN : 50% water: 0.1% TFA(v:v) (ACN50-L)**
Temperature: **Room Temperature**
Column: **Luna C_{18} (Phenomenex):** 4.6 mm ID X 250 mm long (5µm particles)

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).

8.3.2. As an identity test, either use a mass spectrometer to determine the mass associated with the [^{18}F]DCFBC product peak or inject a sample of the [^{18}F]DCFBC product spiked with sufficient DCFBC standard to observe the UV absorbance from the standard. Either the mass spectrum must show the mass (M/Z) to be ~ 402 M/Z (M-H) (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. (A reference standard curve can be used if the investigational site has documented consistency of the standard response curve and a single standard run on the day of the test falls correctly on the standard curve). Two more product injections will follow to assure the product DCFBC chemical analysis obtained from the first injection was correct.

8.4. Data Analysis

8.4.1. Integrate all of the UV and, if applicable, MS responses to obtain the peak areas for the DCFBC standards, and the UV or MS responses to obtain the peak areas for the DCFBC.

8.4.2. Determine the equation for the line of regression between the UV or MS 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 re-run and an OOS investigation initiated.

8.4.4. Perform the reverse correlation to determine the concentration of DCFBC in the final solution.

8.4.5. Typically the final concentration of the [^{19}F]DCFBC in the [^{18}F]DCFBC product to be tested will be < 0.23 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 0.23 µg per mL 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 220 nm UV absorbing chromatographic peaks that elute after more than 5.0 minutes up to 20 minutes post injection, e.g. post injection and mobile phase disturbances, should be integrated individually and shown to sum to less than "15 µg" in the final product. Assume that the molar UV absorption coefficients for the unknown contaminants are the same as for DCFBC and that the DCFBC 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]DCFBC peak.

ACCEPTANCE CRITERIA

Radiochemical Purity: **≥ 90%**

Identity of radioactive peak:
Confirmed by co-injection of [^{19}F]DCFBC or 402 M/Z(ES$^-$)

Sum of UV (220 nm) absorbing contaminants:
≤ 45 µg in the final product dose

Nonradioactive DCFBC (UV 220 nm or 402 M/Z (ES):
≤ 180µg in the final product dose

Sample chromatograms are presented in Figures 1-3. Figure 1 shows an UV absorbance from a chromatogram of [^{19}F]DCFBC standard. Figure 2 shows chromatograms from an [^{18}F]DCFBC product analysis. Figure 3 shows a chromatogram for the DCFBC precursor. Because of the huge difference in lipophilicity, the organic precursor (targeting ligand, SS-PEP) does not elute with [^{18}F]DCFBC product when using 30% acetonitrile-phosphate (**PHOS30-L**) buffer as preparative mobile phase during purification. Precursor can only be seen using totally different HPLC set-up (mobile phase: 5% ACN: 95% water:0.05% H_3PO_4). The chromatograms in figures 1-2 used the methods including mobile phase, 50% :50%:0.1% (water:acetonitrile:TFA) and Luna C_{18} column described in this procedure.

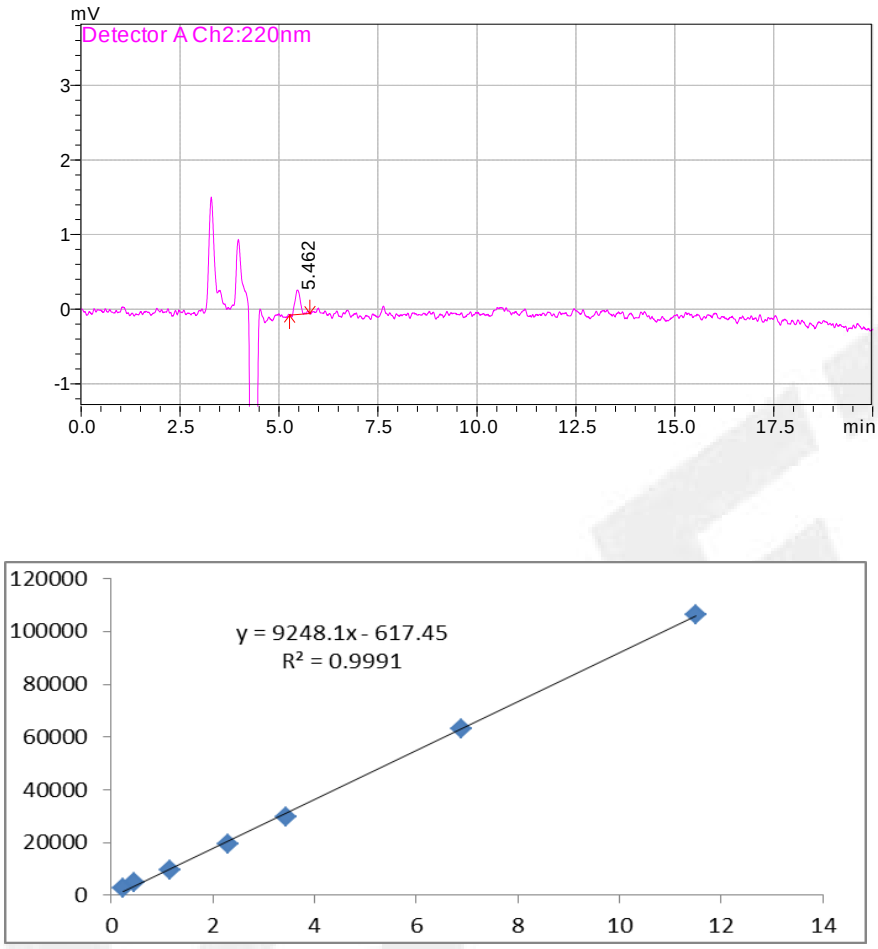

Figure 1. HPLC chromatogram (top) for a 10 µL injection of a 0.23 µg/mL of DCFBC standard at 220 nm with a flow rate of 0.6 mL/min. Typical standard calibration curve (bottom) developed by different concentration (0.23 µg/mL to 11.5 µg/mL) of DCFBC . X-axis represents µg/mL of non-radioactive DCFBC standard. Y-axis represents area of the UV peak (220 nm).

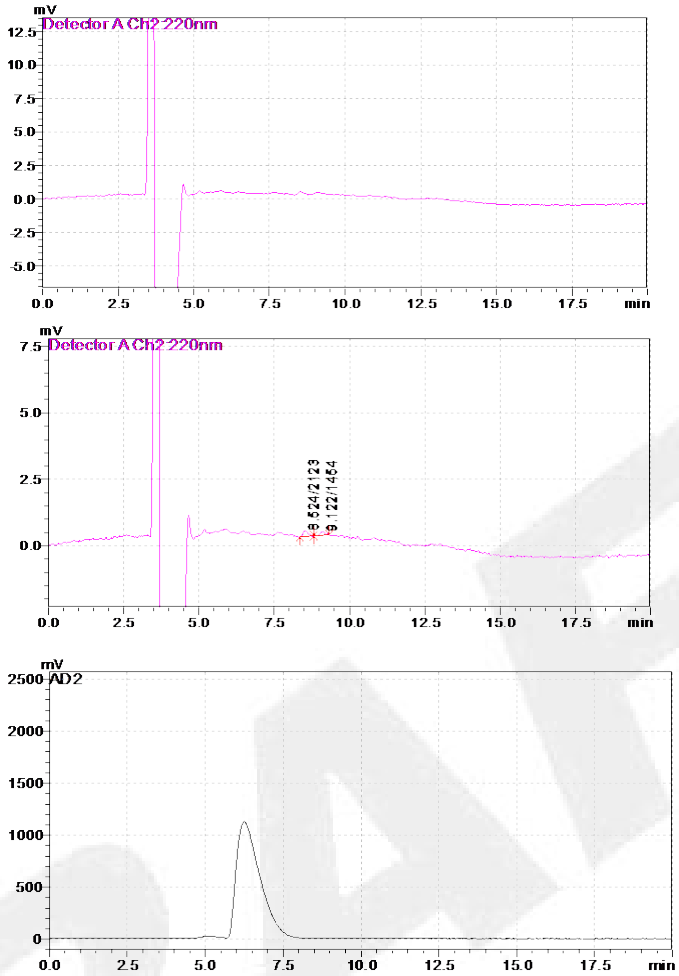

Figure 2 Typical HPLC chromatogram of [^{18}F]DCFBC product. Top trace is the UV absorbance of the product at 220 nm. Middle trace is the expansion of UV chromatogram to detect trace amount of UV impurities. The bottom trace is radioactivity. Minor impurities in the mobile phase and formulation account for the first few peaks seen with the UV at ~ 4.5 min.

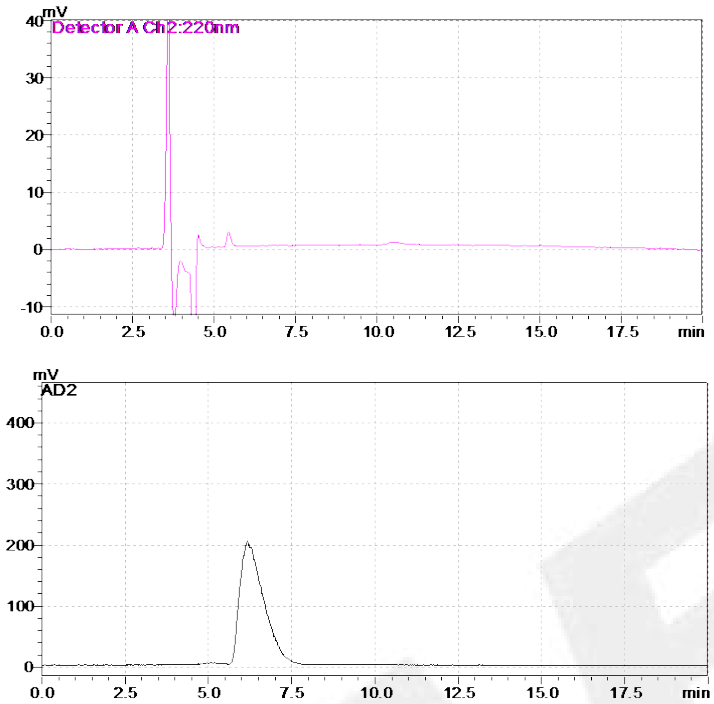

Figure 3 Typical HPLC chromatogram of [^{18}F]DCFBC product co-injection with standard DCFBC. Top trace showed UV absorbance (R_t 5.5 min) at 220 nm and bottom trace showed radiation at 5.8 min. There is tubing between the UV and radiation detectors so that the DCFBC is eluted at 5.5 min for UV and 5.8 min for radiation.