Form-Q321
Standard Operating Procedure of HPLC for ^{89}Zr-Protein

1. **Purpose:** To describe the quality control procedure for using HPLC to determine the mass concentration, chemical and radiochemical purity of the final [^{89}Zr]Protein product solution.

2. **Scope:** The HPLC method described here will be used for determining the chemical and radiochemical purity of the final product, at all manufacturing [^{89}Zr]Protein under [Insert Your Institution Name 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:** 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
   - **4.4** **Form-Q015**, "Standard Operating Procedure 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   - **4.5** FDA Draft Guidance PET Drug Products—Current Good Manufacturing Practice (CGMP)—September 2005 (CDER)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** **PBS-HPLC-L,** "Master Compounding Record for Preparation of Analytical HPLC Mobile phase for ^{89}Zr-Protein"
   - **4.7** **Form-Q119** SOP for concentration determination of DFO-PAN by HPLC
   - **4.8** **Form-Q320** SOP for HPLC of DFO-protein
   - **4.9** **Form-Q118,** "Standard Operating Procedure for Determination of Concentration of DFO-Protein by HPLC"
   - **4.10** **Form-M120,** "Standard Operating procedure Documentation of Manufacturing Variances"

5. **Forms**
   - **Form FQC-005**, "[^{89}Zr]Protein Final Product QC Test Results" Form
   - **Form Q319**, "Preparation of Protein Standard Solution and Dilutions Form"

6. Policies

N/A

7. Materials and Equipment

7.1. Analytical HPLC Column Superdex TM 200 10/300GL, (GE Healthcare) or equivalent. (**SS-Super**)

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.

7.3. **PBS-HPLC-L,** "Master Compounding Record for Preparation of Analytical HPLC Mobile phase for ^{89}Zr-Protein"

7.4. Automatic pipettors with disposable tips and glass graduated cylinders of appropriate sizes

7.5. Glass volumetric flasks and glass or plastic vials for dilutions

8. Procedures

8.1. The retention time for ^{89}Zr-Protein is approximately 15 minutes. Sample chromatograms are shown in Figures 1. All standards must be baseline resolved (resolution > 1.5) for a valid analysis.

Parameters

UV Detector: **Set to 280 nm**

Flow Rate: **0.8 mL/min**

Eluent: **PBS-HPLC-L**

Temperature: **Room Temperature**

Column: **Superdex TM** 200 10/300GL, (**SS-Super**)

8.2. Preparation and Analysis of Sample

8.2.1. A blank injection has been finished before the sample injection to make sure that no UV or/and radiation impurities from the column or/and the instrument.

8.2.2. Inject the sample. (This must be done in triplicate).

8.2.3. 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 ^{89}Zr-Protein chemical analysis obtained from the first injection was correct.

8.3. Data Analysis

8.3.1. Integrate all of the UV and radiation responses to obtain the peak areas for the [^{89}Zr]-Protein.

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8.3.2. Perform the reverse correlation from the linear equation of DFO-Protein (**Form -Q118)** to determine the concentration of [^{89}Zr]-Protein in the final solution.

8.3.3. Typically the final concentration of the [^{89}Zr]-Protein in the [^{89}Zr]-Protein product to be tested will be < 0.2 mg/mL.

8.3.4. Any other 280 nm UV absorbing chromatographic peaks should be integrated individually and shown to sum to less than "5%" in the final product. Assume that the molar UV absorption coefficients for the unknown contaminants are the same as for [^{89}Zr]-Protein and that the [^{89}Zr]-Protein standard curve is applicable to the unknown contaminants.

8.3.5. Integrate all of the radioactive peaks in the chromatogram and calculate the percent of the radioactivity of [^{89}Zr]-Protein in the final product. The radioactivity of [^{89}Zr]-Protein in the final product should be more than 95%.

ACCEPTANCE CRITERIA

Radiochemical Purity: **≥ 95%**
Chemical (UV) Purity: **≥ 95%**
Sum of UV (280 nm) absorbing contaminants: **< 5% in the final product dose**

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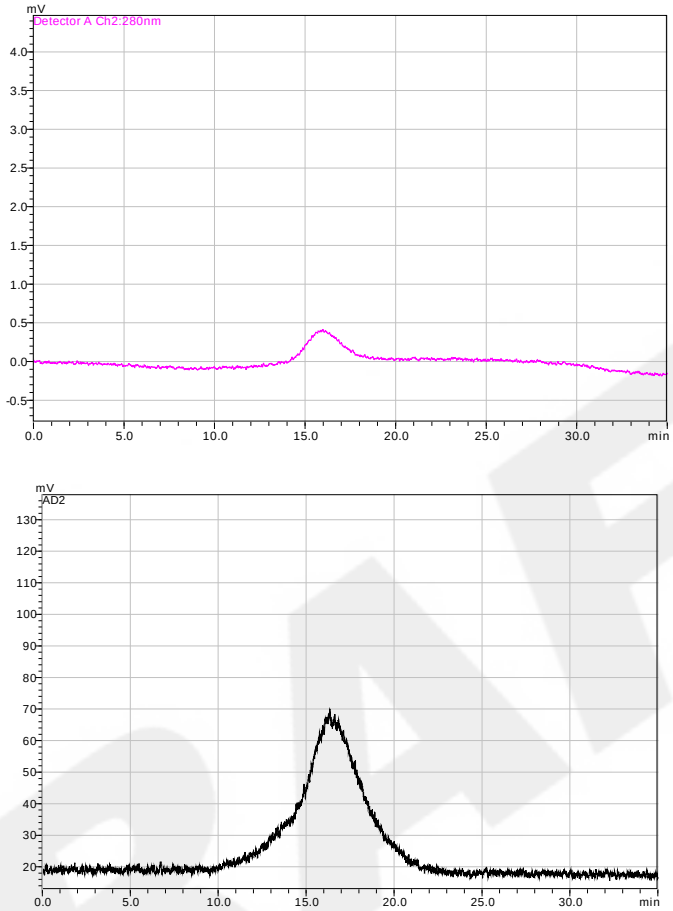

Figure 2. Typical HPLC chromatogram of [^{89}Zr]-Protein product. UV absorbance at 280 nm is shown in the top trace and the bottom trace is the radioactivity. There is tubing between the UV and radiation detectors so that the [^{89}Zr]-Protein product is eluted at ~16 min for UV, ~17 min for radiation.

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