Form-Q322

Standard Operating Procedure Radio-Thin layer Chromatography Analysis

1. **Purpose:** To describe the quality control procedure for determining the radiochemical identity and purity of [^{89}Zr]-Protein Injection by Radio-Thin Layer Chromatography Analysis. The purpose of this assay is meant to measure any unbound Zr-89 in the solution after PD-10 column purification and it is a complement to the HPLC analysis.

2. **Scope:** The Radio-Thin Layer Chromatography Analysis procedure is one of the methods used for determining the radiochemical identity and purity of the final product at all of the contract sites manufacturing [^{89}Zr]-Protein under [Insert your institution name here] IND. This quality control procedure is one of the QC release tests performed on the final product and it must be completed prior to releasing the batch of 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. K. Bauer, L. Gros, W. Sauer. Thin Layer Chromatography: An Introduction. EM Science, Heidelberg, Germany.
   - 4.5. **Form-Q015**, "Standard Operating Procedure Investigating Out of Specification QC Test Results"
   - 4.6. FDA Draft Guidance PET Drug Products—Current Good Manufacturing Practice (CGMP)—September 2005 (CDER)

5. **Forms**

SOP**: Form-Q322** Version: **V1**

Effective Date**:**_____ Supersedes**: None**

Printed Name	Signature	Date
Author:		
Regulatory Approval:		

Procedure becomes effective on latest date of the two approval signatures above. Procedure applies to [^{89}Zr]-Protein IND.

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FQC-005, "[^{89}Zr]-Protein Final Product QC Test Results"

6. Policies

6.1. ITLC plates should be stored with a desiccant in well-closed containers.

6.2. Date stock bottles of ITLC eluent solvent after preparation. It is good for over a year if kept sealed when not in use.

6.3. Always observe radiation safety rules. Check forceps, tweezers, tongs, tubes, and vials for radioactive contamination. Make certain the gamma counter is not contaminated**.**

6.4. If possible, use a counting rate between 10,000 and 200,000 counts per minute for the highest activity sample (peak).

6.5. Always label tubes, vials and strip sections to prevent errors.

7. Materials and Equipment

7.1. ITLC plates, Auburn Biostrips, Cat#31ET (**SS-TLA)**

7.2. ITLC mobile phase, (**CITRICA-L)**

7.3. Gamma radioactivity chromatogram scanner, or counter, or Phosphor imager

7.4. Cellophane rape

7.5. Developing chamber (15mL centrifuge tubes)

7.6. Ruler with metric measurements and a soft-lead pencil

7.7. Pipette, or capillary tube (~1 µL of sample needed)

7.8. Counting Tubes (only needed if you are cutting and counting the strips)

7.9. Dedicated QC Laboratory Notebooks for the Contract Sites

7.10. Photocopier (permanent record)

8. Procedures

8.1. Preparation of the ITLC Chamber & Plate

8.1.1. Transfer 0.5mL ITLC eluent, **CITRICA-L**, into the ITLC developing chamber such that the solvent just covers the bottom of the chamber (less than 1 cm high). Cover the chamber and allow the solvent to equilibrate.

8.1.2. The bottom line in the strip is the origin where the sample will be spotted. The top line in the strip is the solvent front, *i.e.*, the maximum distance that the solvent should be allowed to travel on the plate.

8.1.3. Using a soft lead pencil, label the top of the ITLC plate to indicate prepared [^{89}Zr]-Protein sample type. Write the date at the top of the ITLC plate for reference purposes. Refer to Diagram 1 on page 3.

8.2. [^{89}Zr]Protein Sample Application

8.2.1. Spot ~1 µL of the sample onto the ITLC plate at the origin in the middle.

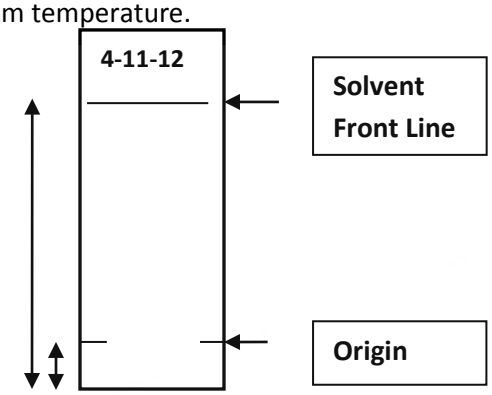
8.2.2. Allow the spots to dry completely prior to placing them in the developing chamber (approximately 5-10 minutes).

8.3. Migration

8.3.1. Develop the ITLC plate, by carefully positioning it within the developing chamber and replacing the lid.

8.3.2. Allow the mobile phase to migrate up the ITLC plate until it just reaches the Solvent Front line (refer to Diagram 1).

8.3.3. Immediately remove the TLC plate from the developing chamber and allow it to dry at room temperature.



NOTE: The radioactive [^{89}Zr]-Protein is not typically be visible.

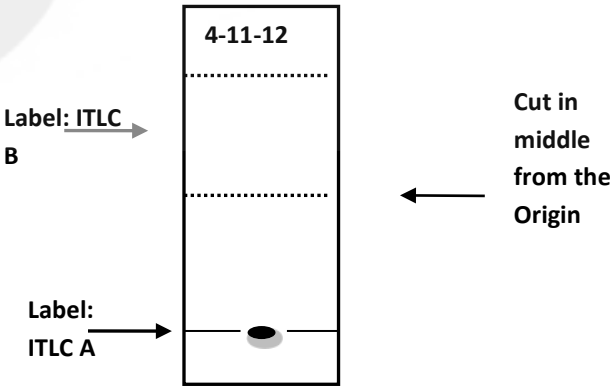
8.4. Methods of Assay for Radiochemical Purity and Identity

Radiochemical purity and identity can be determined by using either a radiochromatogram scanner or by the cutting and counting technique.

8.4.1. Scan or image the developed ITLC plate with an appropriate radiation detecting instrument. Identify all peaks for calculation of radiochemical purity.

8.4.2. Cut the ITLC plate into two sections (Refer to Diagram 2). The sections are transferred into separate labeled counting tubes, and counted for radioactivity with an appropriate radiation counter.

Diagram 2:



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8.5. Calculation of Radiochemical Purity

The % radiochemical purity of the [^{89}Zr]-Protein may be determined in two ways:

8.5.1. One way is to measure the areas under the peaks with a planimeter and using the following equations:

$$\textbf{\% Radiochemical Purity} = \frac{\textbf{Area of Peak at } R_{(< 0.5)}}{\textbf{(Sum of Areas of All Peaks)}} \times 100$$

Where Area of Peak at $R_{(< 0.5)}$ = Area under the peak of the radiopharmaceutical

Area of Peak at $R_{(> 0.5)}$ = Area under the peak of radiochemical impurity of ^{89}Zr

8.5.2. Or by using the count data from the digital output from the scanner unit and calculating the radiochemical purity with the following equation:

$$\textbf{\% Radiochemical Purity} = \frac{\textbf{Counts of Peak at } R_{(< 0.5)}}{\textbf{(Sum of Counts of All Peaks)}} \times 100$$

8.6. Interpretation and Reporting of the QC Results

8.6.1. The Radiochemical purity of [^{89}Zr]-Protein must be ≥ 90% using this assay method.

8.6.2. Record the calculated radiochemical purity results on both the Form **FQC-005**, "[^{89}Zr]-Protein Final Product QC Test Results", and in the QC Laboratory notebook.

NOTE: The final product must meet release specification outlined in 8.6.1 in order for the product to pass this quality control testing requirement.

8.6.3. If the product fails to meet the Radiochemical purity criteria, reject the product and conduct an investigation as per **Form-Q015**, "Standard Operating Procedure Investigating Out of Specification QC Test Results" and record the observations on **FQC-005**, "[^{89}Zr]-Protein Final Product QC Test Results"

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