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Cancer Imaging Program

National Cancer Institute

Standard Operating Procedure

GEN-Q118A

An Alternate Test for the Detection of Kryptofix® [2.2.2]

1. **Purpose:** To describe the quality control procedure for detecting Kryptofix® [2.2.2] in the final product solution.

2. **Scope:** The color spot test described in this procedure will be performed at the sites manufacturing radiopharmaceuticals. It will be used to verify that the residual Kryptofix® [2.2.2] in the final product is below the 50 µg/mL limit in accordance with the FDA guidelines.

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 28, Fludeoxglucose F-18 Injection
   - **4.2.** USP 28 ‹621› Chromatography
   - **4.4.** USP 28 ‹821› Radioactivity
   - **4.5.** USP 28 ‹823› Radiopharmaceuticals For Positron Emission Tomography—Compounding
   - **4.6.** **GEN-Q015**, "Investigating Out of Specification QC Test Results"
   - **4.7.** FDA Draft Guidance PET Drug Products—Current Good Manufacturing Practice (CGMP)—September 2005 (CDER)

5. **Forms**
   - **Form FQC-###**, Final Product QC Test Results
   - **Form IOS-001**, "Investigating Out of Specification (OOS) QC Test Results Form"

SOP**: GEN-Q118A** Date:

Printed Name	Signature	Date
Author:		
Regulatory Approval:		

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6. Policies

6.1. The FDA has proposed a maximum permissible level of 50 µg/mL of K-[2.2.2] in 2-[^{18}F]FDG, therefore this is the maximum permissible level that we are using for the final product.

6.2. Date opened stock bottles of solvents upon receipt and when they are first opened for use. As a general rule, any unused solvents should be discarded after 6 months. Keep a sealed bottle in inventory as a back-up. The purity of solvents that are used as mobile phase is important to ensure reliability of test results. Contaminants, such as water or other chemicals, can alter the system and give inaccurate results. Solvents can evaporate, absorb moisture from the air, or become contaminated with other solvents.

6.3. Always observe radiation safety rules. Check forceps, tweezers, tongs, tubes, and vials for radioactive contamination.

6.4. TLC plates should be stored with a desiccant in well-closed containers.

6.5. Always label tubes and vials to distinguish between solvent types, samples, standards, and strip sections.

7. Materials and Equipment

7.1. Kryptofix® [2.2.2] (Aminopolyether) (**SS-KRY**)

7.2. Iodine (**SS-IOD**)

7.3. Aluminum-backed Silica gel plates, Merck 0.2 mm, F_{254} (size 5 cm x 20 cm or 5 cm x 7.5 cm) (**SS-TLA**)

7.4. Dedicated QC Laboratory Notebooks for the Contract Sites

7.5. Syringe, pipette, or capillary tube (1-2 µL of sample needed)

7.6. Two Developing Chambers

7.7. Product-Specific Solution Matrix

7.8. Ruler with metric measurements

7.9. Soft-Lead Pencil

7.10. Glass container with Teflon® Lined Cap

7.11. Calibrated Balance

7.12. 30% Ammonium Hydroxide (**SS-NHO**)

7.13. Methanol (**SS-MTH**)

7.14. Beta or Gamma Radioactivity counter

8. PROCEDURES

8.1 Preparation of Kryptofix® Standard

8.1.1 Weigh out 5 mg of Kryptofix® in a glass vial, with a Teflon®-lined cap.

8.1.2 Add 1 mL of ethanol to make a 5 mg/mL stock solution and mix thoroughly.

8.1.3 Label the vial as 5 mg/mL of Kryptofix® Stock Solution.

8.1.4 Store this stock solution at 2-8°C in a dark container protected from light until ready to use.

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8.1.5 When ready to use transfer 10 µL of the Kryptofix® Stock Solution into a glass vial with a Teflon®-lined cap and add 1 mL of the appropriate product-specific solution matrix and mix thoroughly. Label this vial as the 50 µg/mL Kryptofix® Standard Solution. Protect this solution from the light as well by placing aluminum around the container.

8.2 Developing Solvent System

8.2.1 Prepare a 9:1 solution of methanol and 30% ammonium hydroxide (v:v) in a sufficient volume to cover the bottom of the TLC chamber, and mix well.

8.2.2 Pour the solution into the TLC chamber. Place the cover on the TLC chamber to allow the developing solvent to equilibrate.

8.3 Preparation of the Plate

8.3.1 Using a soft lead pencil and a ruler, lightly draw tick marks at the edges of the plate and 1 cm up from the bottom of the plate. Do not draw any lines across the plate, as that may disturb the solid phase and interfere with progression of the mobile phase, refer to Diagram 1. This is the origin where the sample and standards will be spotted.

8.3.2 Draw a second line with the pencil 6.5 cm from the bottom of the TLC plate. This is the solvent front, *i.e.*, the maximum distance that the solvent should be allowed to travel on the plate.

8.3.3 Using a soft lead pencil, label the top of the TLC plate to indicate the channels where the freshly prepared final product sample (X) and Kryptofix® standard (S) will be located. Write the date at the top of the TLC plate for reference purposes (as illustrated in the figures).

8.4 Final Product Sample & Kryptofix® Standard Application

8.4.1 Spot approximately 1 µL of the sample onto the TLC plate at the origin below the (X).

8.4.2 Spot approximately 1 µL of the Kryptofix® standard onto the TLC plate at the origin below (S).

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

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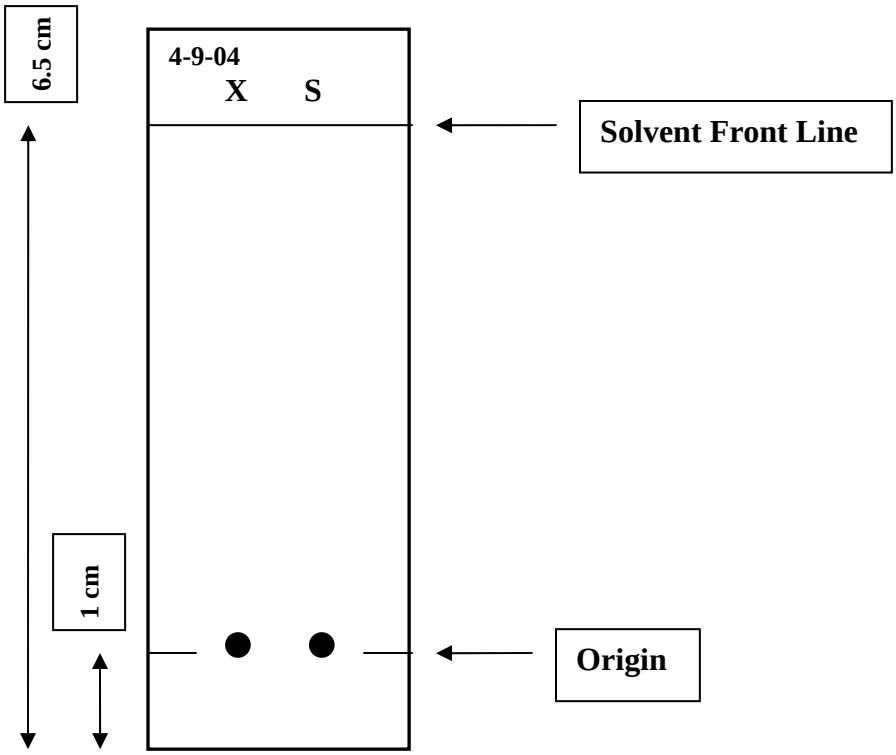

Diagram 1: Preparation

8.5 Migration

8.5.1 Develop the TLC plate, by carefully positioning it within the developing chamber and replacing the lid.

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

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

8.5.4 Once the TLC plate has dried completely, place the TLC plate in a chamber containing iodine crystals.

8.6 Development in Iodine Crystals

8.6.1 Develop the plate in the chamber containing the iodine crystals until a spot is visible on the chromatogram of the Standard Solution of the Kryptofix®.

8.6.2 Once the standard is visible, remove the TLC plate and compare the "test solution" of the final product with that of the standard.

8.6.3 Note in the quality control laboratory notebook whether or not the size and intensity of the spot obtained from the test solution does or does not exceed that obtained from the Standard Solution, Kryptofix®.

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8.7 Interpretation and Reporting of the QC Results

8.7.1 If the size and intensity of the spot obtained from the test solution, final product, is *less than* that of the standard, Kryptofix® Solution, then the sample *passes* the specification for the Kryptofix® test.

8.7.2 If the size and intensity of the spot obtained from the test solution, final product, is *equal to or greater than* that of the standard, Kryptofix® Solution, then the sample *fails* the specification for the Kryptofix® test.

8.7.3 Record the results of this test for the final product sample on both, the **Form FQC-###**, Final Product QC Test Results and in the QC Laboratory notebook.

NOTE: The final product must meet the release specification outlined in 8.7.1 in order for the product to pass this quality control testing requirement.

8.7.4 If the product fails to meet the Radiochemical purity criteria, reject the product and conduct an investigation as per **GEN-Q015**, "Investigating Out of Specification QC Test Results, and record the results of the investigation on the **Form IOS-001**."

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