# Radiochemical purity and patient preparation: Key factors affecting the accuracy of $^{68}$Ga-DOTATOC PET/CT imaging in neuroendocrine tumors

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**Abstract**

Gallium-68-DOTA-D-phe1-try3-octreotide ($^{68}$Ga-DOTATOC) positron emission tomography/computed tomography (PET/CT) is a crucial diagnostic tool for neuroendocrine tumors (NET). Its accuracy is influenced by radiochemical purity and patient preparation. We present two cases where unexpected radiotracer uptake in $^{68}$Ga-DOTATOC PET/CT imaging was observed. One case involved high vascular activity and reduced tumor-to-background contrast, attributed to radiochemical impurities. The other case demonstrated blocked somatostatin receptors due to inadequate patient preparation. Both cases highlight the importance of strict radiolabeling protocols and quality control measures, such as thin-layer chromatography (TLC), to ensure accurate $^{68}$Ga-DOTATOC PET/CT results. Additionally, proper patient preparation, including discontinuing somatostatin analogues as recommended, is essential for optimizing diagnostic accuracy. By addressing these factors, healthcare providers can improve the effectiveness of $^{68}$Ga-DOTATOC PET/CT in diagnosing and managing NET.

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## Introduction

Neuroendocrine tumors (NET) are slow-growing cancers originating from endocrine or nervous tissues, commonly found in the gastrointestinal tract, lungs, pancreas, liver, thymus, and thyroid [1]. These tumors often secrete hormones, leading to carcinoid syndrome with symptoms like diarrhea and flushing, and result in a diminished quality of life for patients. Neuroendocrine tumors frequently overexpress somatostatin receptors (SSTR), which can be targeted for therapy [2].

The incidence of NET has increased to approximately 8 per 100,000 individuals [3]. Given their often subtle initial symptoms, NET are frequently diagnosed at a metastatic stage, significantly affecting prognosis. Survival rates range from 50%-80% for localized cases to 20%-60% for metastatic ones [3, 4].

For imaging, three radiolabeled somatostatin analogues-gallium-68- DOTA-D-phe1-tyr3-octreotate ($^{68}$Ga-DOTATATE), $^{68}$Ga-DOTA-1-Nal3-octreotide (DOTANOC), and $^{68}$Ga-DOTA-D-phe1-try3-octreotide (DOTATOC)-are used. Although they have different affinities for somatostatin receptors (SSTR) subtypes, they offer comparable diagnostic accuracy. Positron emission tomography/computed tomography (PET/CT) imaging with these tracers is crucial for staging NET, with accurate imaging being essential for effective treatment planning and avoiding unnecessary procedures [5].

### Assessing radiochemical purity of radiopharmaceuticals

Radiochemical purity (RCP) is a critical quality control parameter for radiopharmaceuticals, ensuring accurate diagnosis and minimizing potential adverse effects. Radiochemical impurities, while rarely causing serious toxicity, can significantly degrade image quality, increase radiation dose, and lead to incorrect localization, ultimately compromising diagnostic accuracy.

To evaluate RCP, the instant-thin layer chromatography (ITLC) method is widely employed in radiochemistry. Instant-thin layer chromatography is a rapid and high-resolution technique capable of analysing small samples of various radiopharmaceuticals, making it versatile for clinical applications [6].

The European Association of Nuclear Medicine (EANM) has recently published recom-

mendations for validating analytical methods to assess radiopharmaceutical quality, emphasizing the importance of quality assurance in radiopharmacy and the need for validated radiopharmaceuticals for human use [7, 8].

## $^{68}$Ga generator production

Gallium-68-based radiopharmaceuticals have emerged as valuable tools for imaging neuroendocrine tumors (NET) due to their ability to target SSTR. The production of $^{68}$Ga involves the use of a germanium-68 ($^{68}$Ge)/$^{68}$Ga generator, a specialized device that separates the desired $^{68}$Ga isotope from its parent isotope, $^{68}$Ge. The $^{68}$Ge/$^{68}$Ga generator typically consists of a solid-phase matrix, such as titanium dioxide or tin dioxide, and a hydrochloric acid (HCl) solution. Germanium-68, with a half-life of 271 days, is immobilized within the matrix. As $^{68}$Ge decays, it releases $^{68}$Ga, which can then be eluted from the generator using hydrochloric acid (HCl).

The eluted $^{68}$Ga solution is highly enriched with the desired isotope, with minimal contamination from foreign ions such as iron, copper, and zinc. These impurities can interfere with the labeling efficiency of $^{68}$Ga with the specific targeting molecule, such as DOTA-octreotide, used in NET imaging. To prevent the accumulation of impurities, particularly zinc ions, it is recommended to elute the $^{68}$Ga generator within 48 hours of the previous elution. This helps to minimize the buildup of zinc ions, which can compete with $^{68}$Ga for binding sites on the targeting molecule. The $^{68}$Ge/$^{68}$Ga generator is typically stored in a shielded area within the nuclear medicine department to protect personnel from radiation exposure. The generator is designed to provide a continuous supply of $^{68}$Ga for radiopharmaceutical production, enabling frequent and efficient preparation of imaging agents for NET patients [9-11].

## Patient preparation

Before undergoing a SSTR PET scan, patients should follow specific preparation guidelines to ensure accurate imaging results. These guidelines may vary depending on the individual's clinical situation and the specific radiotracer used. However, general recommendations include:

- **Discontinuing short-acting somatostatin analogues (SSA):** Patients should avoid taking short-acting SSA for at least 12 hours prior to the SSTR PET scan. This is to prevent interference with the radiotracer's ability to bind to SSTR [12].
- **Scheduling SSTR PET before long-acting SSA dosing:** If patients are receiving long-acting SSA, the SSTR PET scan should ideally be scheduled before the next dose. This helps minimize potential SSTR blockade and improve image quality [12].
- **Discontinuing long-acting SSA for a specified period:** For patients receiving long-acting SSA, it may be necessary to temporarily discontinue their medication for 3-4 weeks before the SSTR PET scan. This allows for sufficient clearance of the long-acting SSA from the body and reduces the risk of SSTR blockade [13].

## $^{68}$Ga-DOTATOC kit preparation

Gallium-68-DOTATOC is a widely used radiotracer for positron emission tomography (PET) imaging of neuroendocrine tumors that express somatostatin receptors [9, 10]. It is prepared by radiolabelling DOTA-tyr3-octreotide with $^{68}$Ga, a positron-emitting radionuclide produced from a $^{68}$Ge/$^{68}$Ga generator.

Kit-based preparation of $^{68}$Ga-DOTATOC offers several advantages, including ease of use, reduced preparation time, and improved reproducibility. The Somakit TOC kit is a commercially available option that simplifies the radiolabelling process.

## Here's a step-by-step guide for preparing $^{68}$Ga-DOTATOC using the somakit TOC kit:

**1. Prepare the reaction mixture:**

**Figure 1.** Galli Ad generator at NM department of Theageneio Cancer Center.

- Add the appropriate volume of reaction buffer (vial 2) to the lyophilized precursor (vial 1) to establish the desired pH (typically 3.2-3.8).
- Connect vial 1 to the $^{68}$Ge/$^{68}$Ga generator and elute the $^{68}$Ga solution into the vial.

2. **Radiolabelling:**
   - Place the vial containing the reaction mixture in a preheated dry bath at 95°C for 8-10 minutes. This allows the $^{68}$Ga to chelate with the DOTA-tyr3-octreotide.
3. **Quality control:**
   - Perform thin-layer chromatography (TLC) to assess the radiochemical purity of the $^{68}$Ga-DOTATOC. The labelling efficiency should be greater than 95%.
   - Visually inspect the solution for any particulate matter or discolouration.
4. **Formulation and administration:**
   - Dilute the $^{68}$Ga-DOTATOC solution with sterile saline to the desired concentration and volume for injection.
   - Administer the radiotracer to the patient according to the prescribed dose and imaging protocol.

**Key considerations:**

- **pH:** Maintaining the pH within the recommended range is crucial for efficient radiolabelling and optimal radiochemical yield [11].
- **Temperature:** Heating the reaction mixture to the specified temperature ensures complete chelation of $^{68}$Ga with the DOTA-tyr3-octreotide.
- **Radiochemical purity:** High radiochemical purity is essential for accurate PET imaging and to minimize radiation exposure to the patient.
- **Aseptic technique:** Maintain aseptic conditions throughout the preparation process to prevent contamination and ensure the sterility of the final product.

## Thin layer chromatography

Thin layer chromatography (TLC) is a widely used technique to assess the radiochemical purity of $^{68}$Ga-DOTATOC, a radiotracer employed in positron emission tomography (PET) imaging of NET. Radiochemical purity is a critical quality control parameter that ensures the accuracy of diagnostic results and minimizes potential side effects [7].

The TLC procedure involves applying a small sample of the $^{68}$Ga-DOTATOC solution to a strip of glass-fiber ITLC paper, followed by its placement in a developing tank containing a mobile phase (sodium citrate 0.1M, pH 5, in water). The mobile phase moves along the paper, separating the radiotracer from any free $^{68}$Ga ions. After development, the strip is scanned using a radiometric ITLC scanner to quantify the radioactivity at different positions (Figure 2) [8].

The labelling efficiency of $^{68}$Ga-DOTATOC should be >98% to ensure high-quality PET images. The area under the curve obtained from the TLC scan provides the percentage of radiochemical purity, with an acceptable threshold typically set at <2% for free $^{68}$Ga [11]. By performing TLC analysis, radiochemists can:

- Verify the successful radiolabeling of $^{68}$Ga-DOTATOC.
- Identify and quantify any radiochemical impurities.
- Ensure the quality and safety of the radiotracer for clinical use.

In conclusion, TLC is an essential tool for quality control in the production and use of $^{68}$Ga-DOTATOC, contributing to the accuracy and reliability of PET imaging in the diagnosis and management of neuroendocrine tumors.

## Biodistribution of $^{68}$Ga-DOTATOC PET/C

Gallium-68-DOTATOC is a radiopharmaceutical specifically designed to target somatostatin receptors, primarily SSTR2, in the body. This receptor is overexpressed in various neuroendocrine tumors (NET) [14]. The normal physiological distribution of $^{68}$Ga-DOTATOC is influenced by both the presence of these receptors and non-specific clearance mechanisms (Figure 3). Understanding this distribution pattern is crucial for interpreting PET/CT scans and identifying abnormal areas of increased uptake that may indicate the presence

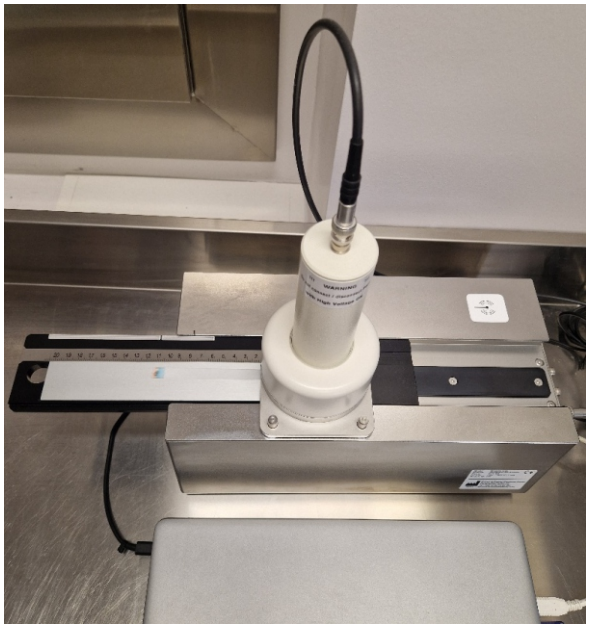

**Figure 2.** Instant thin layer chromatography scanner.

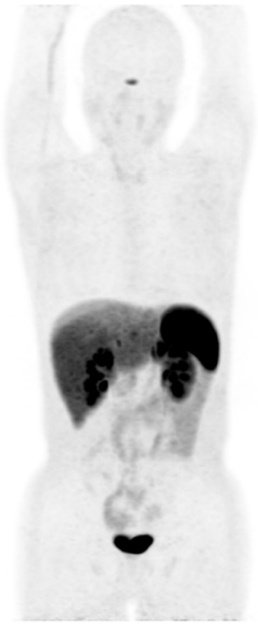

**Figure 3.** Normal distribution of $^{68}$Ga-DOTATOC includes prominent activity in the spleen and kidneys, increased uptake in pituitary gland and adrenals, moderate uptake in liver and variable uptake in pancreatic head, thyroid, salivary glands, stomach wall and prostate gland. Urinary excretion is again seen along with variable bowel activity.

of neuroendocrine tumors.

Here's a breakdown of the key areas of uptake:

**High uptake:**

- **Spleen:** Due to the high density of SSTR2 receptors in the red pulp.
- **Adrenal Glands:** These glands naturally express SSTR2.
- **Kidneys:** The kidneys are involved in the elimination of the tracer.
- **Pituitary Gland:** This gland also expresses SSTR2.

**Moderate uptake:**

- **Liver:** The liver plays a role in the metabolism and clearance of the tracer.
- **Thyroid Gland:** While not a primary target, the thyroid gland may show some uptake due to the presence of SSTR2.
- **Salivary Glands:** These glands may also exhibit moderate uptake.

**Low uptake:**

- **Lungs:** The lungs have a low expression of SSTR2 receptors, leading to minimal tracer uptake, as there are mainly SSTR4 receptors present in the lungs.
- **Prostate Gland:** While SSTR2 receptors are present in the stromal tissue of the prostate, the uptake is typically low and uniform.
- **Gastrointestinal Tract:** The fundus of the stomach and intestinal mucosa may show some activity due to SSTR2 expression.
- **Bone Marrow:** SSTR2 receptors are found on macrophages and hematopoietic precursors, resulting in low-grade activity in the bone marrow.

**Other factors:**

- **"Sink Effect":** In cases of high tumor burden, there may be a decreased uptake in normal tissues due to the tracer being preferentially attracted to the tumor sites.

### Case Reports: Unexpected Radiotracer Uptake in $^{68}$Ga-DOTATOC PET/CT imaging

#### Case 1

A 65-year-old woman with a known history of neuroendocrine tumors underwent a $^{68}$Ga-DOTATOC PET/CT scan. The imaging revealed lower-than-expected radiotracer uptake in the target organs and unexpectedly high tracer activity in the blood vessels, (Figure 4). Pre-injection ITLC analysis showed a radiochemical purity of 1% (<2%) (Figure 5).

#### Case 2

A 55-year-old man also underwent a $^{68}$Ga-DOTATOC PET/CT scan, which demonstrated similar findings of high tracer activity in the vessels and reduced tumor-to-background contrast (Figure 6). However, an ITLC test was not performed in this case.

## Discussion

Gallium-68-DOTATOC PET/CT is a valuable tool for imaging neuroendocrine tumors that express somatostatin receptors. However, the accuracy of this imaging technique can be influenced by various factors, including the quality of the radiolabelled $^{68}$Ga-DOTATOC and patient preparation.

Radiolabelling-related factors can impact image quality. The stringent conditions required for $^{68}$Ga-DOTATOC radiolabelling, such as boiling at 95°C for 8-10 minutes and maintaining a pH of 3.2-3.8, make it susceptible to errors. Deviations from these parameters can lead to incomplete labelling, resulting in the presence of radiochemical impurities [15]. These impurities can interfere with image quality and potentially misdiagnose patients.

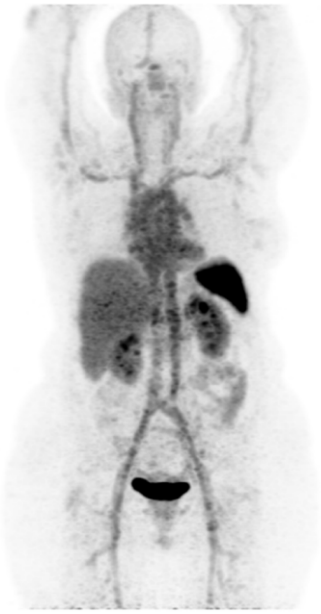

**Figure 4.** Abnormal distribution of $^{68}$Ga-DOTATOC with high tracer activity in the vessels and lower-than-expected radiotracer uptake in the organs physiologically distributed.

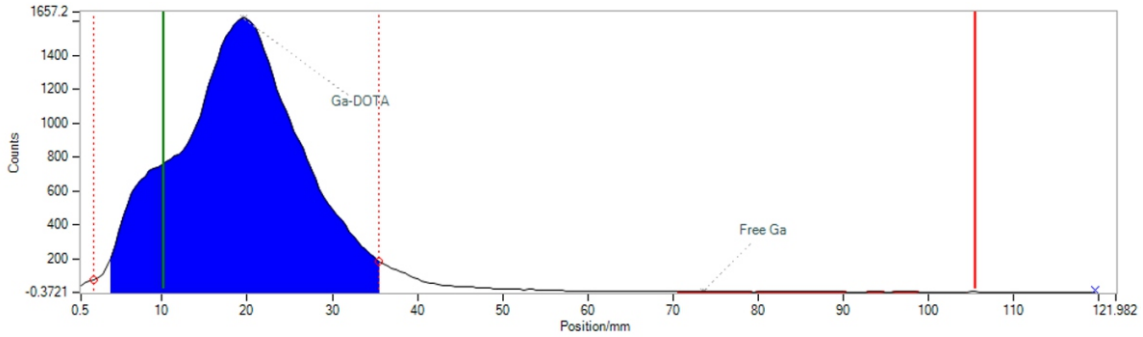


**Figure 5.** The ITLC curve demonstrates the distribution of radioactivity along the glass-fiber paper strip. The main peak (blue) at positions 5-35mm corresponds to the bound $^{68}$Ga-DOTATOC, which moves slower due to its larger size and higher affinity to the stationary phase. The smaller peak at higher positions represents free $^{68}$Ga, which moves further due to its smaller size and lower affinity. The presence of a prominent bound peak and a minimal free $^{68}$Ga peak indicates that the radiolabelling process was successful, with a high yield of $^{68}$Ga-DOTATOC and minimal residual free $^{68}$Ga.

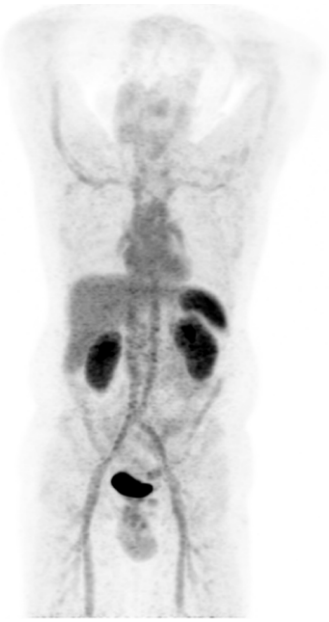

**Figure 6.** Abnormal distribution of $^{68}$Ga-DOTATOC with high tracer activity in the vessels and lower-than-expected radiotracer uptake in the organs physiologically distributed.

***Table 1.*** *Factors Affecting the Accuracy of $^{68}$Ga-DOTATOC PET/CT imaging in neuroendocrine tumors.*

| Factor | Description | Impact on Accuracy |
|---|---|---|
| Radiochemical Purity | The percentage of the radiotracer that is in the desired chemical form. | Low RCP can lead to image artifacts and reduced diagnostic accuracy. |
| Patient Preparation | Adherence to specific guidelines for discontinuing somatostatin analogues and fasting before the scan. | Improper preparation can affect radiotracer uptake and image interpretation. |
| Radiolabeling Technique | Adherence to recommended pH, temperature, and other parameters during the radiolabeling process. | Deviations can lead to radiochemical impurities and reduced image quality. |
| Image Acquisition and Processing | Proper scanner settings, patient positioning, and image reconstruction techniques. | Technical artifacts and noise can affect image quality and interpretation. |
| Clinical Context | Correlating imaging findings with patient history, symptoms, and other diagnostic tests. | Helps to differentiate between benign and malignant findings and guide treatment decisions. |
| Tumor Heterogeneity | Variations in metabolic activity within a tumor. | Can lead to false negative findings if only certain areas of the tumor are detected. |

In our second case, the absence of ITLC testing prevented the detection of radiochemical impurities. This could have contributed to the higher-than-expected radiotracer uptake in the vascular compartment, potentially leading to diagnostic errors. Factors such as deviations from the recommended boiling time or temperature, non-aseptic conditions during the labelling process, and pH variations can all contribute to the presence of impurities [9, 11].

Potential pitfalls associated with PET/CT imaging of NET include both false positive and false negative findings.

False positive findings can arise from various factors, such as:

- Non-malignant conditions: Inflammation, infection, benign tumors, or physiological processes can sometimes mimic the metabolic activity of cancer cells, leading to false positive results [16].
- Image artifacts: Technical artifacts or limitations in image quality can occasionally lead to misinterpretation of findings.

False negative findings often relate to tumor heterogeneity. This occurs when a tumor exhibits varying metabolic activity within different regions. In such cases, fluorine-18-fluorodeoxyglucose ($^{18}$F-FDG) PET/CT can play a complementary role by detecting areas of the tumor that may be dedifferentiated and have increased glucose metabolism.

To minimize these pitfalls and improve diagnostic accuracy, it is important to:

- Consider the clinical context: Correlate imaging findings with patient history, symptoms, and other diagnostic tests.
- Use advanced imaging techniques: Employ techniques like dynamic PET or whole-body PET/CT to enhance image quality and detect subtle changes.
- Consult with experienced radiologists: Seek expert interpretation of imaging studies to minimize errors and optimize patient care.
- Be aware of potential pitfalls: Radiologists should be familiar with the limitations of $^{68}$Ga-DOTATOC PET/CT and consider these factors when interpreting images.

By addressing these potential pitfalls and utilizing best practices in imaging and interpretation, healthcare providers can improve the diagnostic accuracy and clinical outcomes for patients with neuroendocrine tumors.

Patient preparation is another crucial factor affecting the accuracy of $^{68}$Ga-DOTATOC PET/CT. In the case of the 65-year-old woman, the lack of timely discontinuation of long-acting somatostatin analogues (SSA) led to blocked somatostatin receptors, hindering the ability of $^{68}$Ga-DOTATOC to bind to tumor cells [12]. This resulted in unexpected radiotracer uptake in non-target areas, complicating the diagnostic process.

*In conclusion*, adhering to strict radiolabelling protocols and performing thorough quality control measures, such as ITLC testing, is essential to ensuring accurate and reliable results with $^{68}$Ga-DOTATOC PET/CT. Additionally, proper patient preparation, including discontinuing SSA as recommended, is crucial for optimising the diagnostic accuracy of this imaging modality. By addressing these factors, healthcare providers can improve the effectiveness of $^{68}$Ga-DOTATOC PET/CT in diagnosing and managing neuroendocrine tumors.

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