Application Note

Cut and Count vs. radio-TLC scanners

Introduction

Globally, strict limits for radiopharmaceuticals are determined by legislative bodies in each country. In the UK, radiopharmaceutical preparation in hospitals must adhere to the Good Manufacturing Practice (GMP) guidelines, outlined in European Directive 2003/94/EC and incorporated into the Medicines Act 1968 (1).

To ensure Quality Assurance (QA) in radiopharmaceuticals, the UK Radiopharmacy and NHS Pharmaceutical Quality Assurance Committee have jointly developed comprehensive guidelines (2). These guidelines cover various aspects of QA, including radiochemical purity (RCP) testing. Regular testing of radiopharmaceuticals is recommended for all radiopharmacies, and these guidelines serve as the minimum standards during inspections conducted by the Medicines and Healthcare Products Regulatory Agency (MHRA).

Furthermore, the European Commission's "Good Manufacturing Process Vol. IV" guide establishes minimum standards for facilities, personnel, and quality systems required for commercial manufacturers to market such products (3). Also, the Pharmaceutical Inspection Convention and Pharmaceutical Inspection Cooperation Scheme (PIC/S) have defined standards specifically for hospital preparation of radiopharmaceuticals (4).

Due to an increasing number of regulations, the awareness around the importance of radiochemical purity (RCP) testing has increased. To understand the significance of radiochemical purity, it is essential to define what it and explain why it holds such prominence in the field of Nuclear Medicine.

Radiochemical purity

Low radiochemical purity is a common occurrence in radiopharmacies, arising from incomplete labelling, breakdown of radiolabelled preparations, or through introduction of contaminants during the synthetic process. These radiochemical impurities can hinder clinical interpretation by decreasing the target to non-target ratio resulting in the patient returning to the department for a repeat scan culminating in an additional radiation dose for the patient. If a radiopharmaceutical is used for therapy, a low RCP could mean unacceptable radiation dose to healthy organs and tissues through altered biodistribution. As a consequence, to bring a radiopharmaceutical such as FDG (F-18) to the market, the lower limit of RCP is 95%, that is, at least 95% of the radioactive isotope must be attached correctly to the ligand.

Why is RCP testing important ?

RCP testing provides assurance of purity at the time the product leaves the radiopharmacy. According to the UK guidance (2), RCP testing is a minimum standard required for off label manufacture. An example of this is when too high an activity is inadvertently added to a kit. In times of ^{99}Mo molybdenum shortage, discarding what could be a kit with perfectly satisfactory radiolabelling could result in cancellations of appointments and delays in diagnosis and treatments. An RCP test before release could support the use of the product and makes the decision to release it an objective one rather than a subjective one.

Furthermore, a cost-effectiveness study conducted by Ponto and Ponto (5) revealed that the direct cost of RCP testing was lower than the direct cost of preparing replacement doses in the event of product failures. This finding serves as evidence against the claim that RCP testing imposes unnecessary expenses. In reality, greater expenditure would be required to replace failed products than to test them initially.

Therefore, given the substantial body of evidence supporting RCP testing, understanding the underlying reasons people are against it is important.

Cut and count

The SmCP (Summary of Product Characteristics) endorses cut and count as the primary method for RCP testing (6). It involves segmenting a chromatography strip and measuring radioactivity in each segment individually using a calibrator or scintillation counter. This allows for quantifying radioactivity levels and manually calculating activity percentages in specific sections.

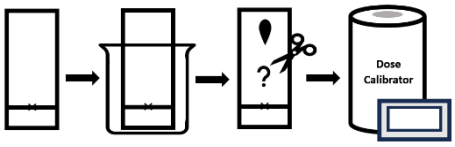

Figure 1 - Schematic of the cut and count process

A crude and inaccurate technique

A survey of 26 UK radiopharmacies revealed a surprising widespread opposition to cut and count. Staff expressed concerns on increased personal radiation exposure and contamination risks when handling chromatograms with known radioactivity. They criticised the technique as "crude and inaccurate" with statistically invalid sampling (7).

Assuming chromatographic separation

Cut and count often makes assumptions about chromatographic separation and substance distribution leading to false negative and false positive results (as shown in Figures 2 and 3 below). Manual Thin Layer Chromatography (TLC) suffers from limited resolving power, making it inadequate for comprehensive compound identification. This often necessitates multiple methods, needlessly prolonging the process. Time constraints can be an issue, with some methods taking 20-30 minutes or longer.

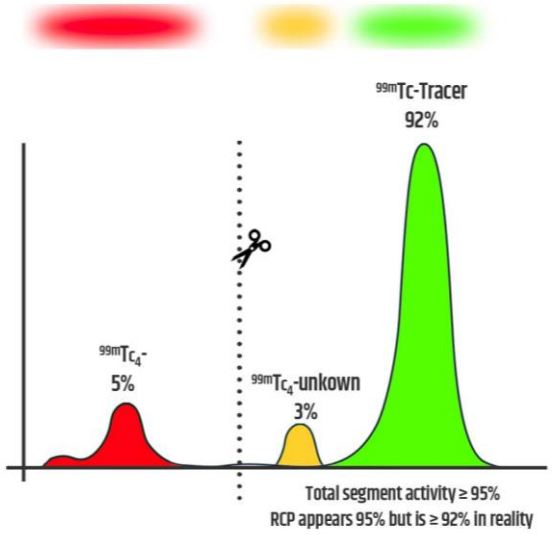

Figure 2 - False positive result due to unknow chemical impurity

Chemical impurities in the radiolabelled compound can affect cut and count accuracy. Non-radioactive impurities may cause false positives, while impurities not contributing to the signal may lead to false negatives.

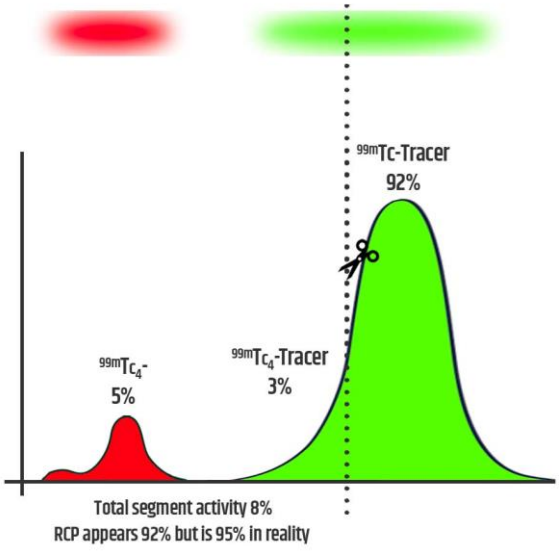

Figure 3 - False negative result due to tailing peak

Tailing peaks in cut and count can result in inaccurate quantification, making it challenging to determine the true area under the curve. Additionally, tailing peaks may overlap with adjacent peaks, hindering accurate identification and quantification of individual components, potentially causing false results in radiochemical purity assessment.

Reduced reproducibility

Radiopharmacists prioritise data integrity and robustness in testing methods. These characteristics are hard to achieve with cut and count due to the inability to reproduce data efficiently. The emergence of radiochromatogram scanners have enhanced the robustness and data integrity of RCP testing, prompting us to explore their specific advantages.

Radio-TLC scanning

TLC chromatograms can be analysed using a radiochromatogram scanner, offering advantages such as reproducibility, reduced radiation exposure, and decreased risk of contamination.

High sensitivity

The radio-TLC scanner is known for its exceptional sensitivity when it comes to detecting radiochemical impurities. Even at low concentrations, this method can detect and quantify impurities, making it an invaluable tool for quality control in the production of radiopharmaceuticals, where stringent purity requirements must be met.

Reduced radiation exposure

In order to reduce personal radiation exposure, radio-TLC scanners are designed to handle radioactive samples remotely. This means that laboratory personnel do not need to come into direct contact with the radioactive material and particularly not cut the TLC strip in pieces. Instead, they can load the samples with a detachable scanner bed onto the scanner and operate it from a safe distance.

Enhanced reproducibility

Reproducibility is a critical aspect of any analytical method, and the radio-TLC scanner excels in this regard. Assuming consistent and controlled conditions of the TLC process, combined with the scanner's ability to provide reliable and repeatable results, results in a robust choice for routine radiochemical purity testing.

Quantitative analysis

Radio-TLC scanners provide quantitative data, allowing for precise calculation of the radiochemical

purity of a sample. This quantitative analysis is crucial for regulatory compliance and quality assurance in various industries, including healthcare and nuclear research.

Conclusion

Given the increasing concern by regulatory authorities, quality assurance and quality control, personnel working in radiopharmacies should seek to constantly update their knowledge and implementation of safter, robust and secure analytical methods for assessing radiochemical purity.

In accordance with UK legislation and guidance, conducting RCP testing before the radiochemical leaves the radiopharmacy is of utmost importance. While RCP testing has faced criticism in the past, recent advancements such as radio-TLC scanners have significantly improved its efficiency, safety, and data integrity.

The radio-TLC scanner stands out as a superior method for testing radiochemical purity due to its, high sensitivity, quantitative analysis capabilities, and enhanced reproducibility. In an era where accuracy and reliability are paramount, this technique has earned its place as a cornerstone of radiochemical analysis in fields ranging from healthcare to nuclear research. As technology continues to advance, the radio-TLC scanner remains a robust and accurate choice for ensuring the quality and safety of radiochemical compounds.

References

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