

# Simple and reliable Chromatographic technique for the quality control of $^{177}\text{Lu}$ -DOTATATE

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## Introduction

A dedicated and reliable quality control procedure (as well as sterility and environmental monitoring) is very important to guarantee the success of therapy trials.  $^{177}\text{Lu}$ -[DOTA<sup>0</sup>,Tyr<sup>3</sup>] octreotate ( $^{177}\text{Lu}$ -DOTATATE) is successfully used in Peptide Receptor Radionuclide Therapy (PRRT) and its quality assurance should be always carefully determined.

Radio-Chemical Purity (RCP), as stated in the European Pharmacopoeia, is an essential parameter ensuring the quality of the radioactive pharmaceutical product.

$^{177}\text{Lu}$ -DOTATATE RCP determination is routinely performed by an analytical technique employing Solid Phase Extraction (SPE).

**Objective** of the current study was to develop another procedure possibly more accurate than SPE. For this reason we set up, in our nuclear medicine lab, a new Thin Layer Chromatographic (TLC) method and compared it to the traditional SPE.

## Materials and Methods

Radiolabeling procedure was carried out at a specific activity ranging between 37-45 MBq  $^{177}\text{Lu}$  /ug of DOTATATE buffered with a solution of sodium acetate and gentisic acid at pH 5.0. Then the mixture was heated for 30 min at 90°C. After labelling,  $^{177}\text{Lu}$ -DOTATATE radiochemical purity was determined by the two analytical techniques employed (TLC and SPE).

TLC method involved the use of Thin Layer Chromatography (TLC) plates with C18 derivatized Silica-Gel (Whatman<sup>®</sup>, Maidstone UK) as stationary phase, developed in ammonium acetate (1M, pH 7): methanol (10:90, v:v) mixture.

An aliquot of the radiopharmaceutical mixed with an excess of EDTA was spotted on C18 TLC strip. After its development in the solvent system, the strip was air-dried and counted for activity. The radiochromatographic profile was determined by an autoradiography system consisting of a laser scanning device and a high performance storage phosphor screen (Cyclone<sup>™</sup>, Packard BioScience, Meriden USA) which provides direct counting of plate without the need for cutting.

The Retention factor (Rf) was determined for each radiochemical species (free radioisotope and  $^{177}\text{Lu}$ -DOTATATE).

About SPE, the procedure involved the use of Sep Pak<sup>®</sup> C18 cartridges (Waters, Milford MA) with methanol and sodium acetate buffer (0.05 M, pH 5.05) as mobile phases. The cartridge was previously conditioned with 2mL of methanol and with 2 mL of sodium acetate; an aliquot of radiolabel solution mixed with an excess of EDTA, was then loaded into the cartridge which was eventually eluted with 2mL of acetate and 2mL of methanol, respectively. The first eluted fraction (acetate buffer) contained free  $^{177}\text{Lu}$  and the second (methanol) contained  $^{177}\text{Lu}$ -DOTATATE.

To compare the two analytical techniques we assayed forty preparations with high and low RCPs.

Radiospecies	Rf values
$^{177}\text{Lu}$ -DOTATATE	0.65±0.03
$^{177}\text{Lu}$ -EDTA	0.85±0.05

**TABLE 1.** Rf values of  $^{177}\text{Lu}$  species according to the chromatographic technique employed.

## Results

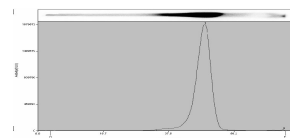
A typical radiochromatogram of  $^{177}\text{Lu}$ -DOTATATE is shown in FIGURE 1. TLC profiles showed sharp and narrow peaks for  $^{177}\text{Lu}$ -DOTATATE and free  $^{177}\text{Lu}$  bound to EDTA. Their Rf values are shown in TABLE 1.

The mean RCPs of this radiopharmaceutical, as determined by the TLC and SPE techniques, are reported in TABLE 2.

The results were comparable when preparations with high RCPs were tested (TABLE 2A). However, this similarity was not observed in the samples with low RCPs as shown in TABLE 2B.

TLC technique gave more accurate results compared to SPE. In fact, adding  $^{177}\text{LuCl}_3$  prior to RCP test, only TLC method was able to detect the excess of free radioisotope whereas in the SPE, part of free Lutetium added was eluted into the methanol fraction.

The time required for the two techniques was similar.



**FIGURE 1.** A typical radiochromatogram of  $^{177}\text{Lu}$ -DOTATATE obtained by TLC technique. RCP > 99%.

A		$^{177}\text{Lu}$ -DOTATATE with high RCPs % (mean ± s.d.)
Analytical procedure employed		
SPE		99.7±0.2
TLC		99.6±0.2
B		$^{177}\text{Lu}$ -DOTATATE with low RCPs % (mean ± s.d.)
Analytical procedure employed		
SPE		85.2±7
TLC		72.9±2.9

**TABLE 2.** Radiochemical purities (mean ± s.d.) determination according to the two analytical procedures (TLC and SPE) employed.

A)  $^{177}\text{Lu}$ -DOTATATE with high RCPs% (n=30);

B)  $^{177}\text{Lu}$ -DOTATATE with low RCPs% (n=10).

## Conclusions

TLC procedure successfully separated  $^{177}\text{Lu}$ -DOTATATE from free Lutetium even when the latter was present in large amount that could be co-eluted in the methanol fraction by SPE, thus originating an inaccurate result. Moreover, the chromatographic method reduces radiation exposure to the operator representing an additional valuable advantage.

The new TLC system proved to be a simple, reliable, fast and accurate method to determine  $^{177}\text{Lu}$ -DOTATATE quality control even with low RCP result and, following further investigations, could be adopted to other radiopharmaceuticals such as  $^{111}\text{In}$  and  $^{90\text{Y}}$  labeled peptides.