Quality control of [^{225}Ac]Ac-DOTA-TATE and [^{225}Ac]Ac-PSMA-D4: a crucial aspect of safe clinical application of alpha therapy

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INTRODUCTION

Targeted alpha therapy (TAT) has emerged as a highly promising approach in nuclear medicine, offering potent cytotoxicity and high selectivity for tumor cells. Among the alpha-emitting radionuclides, actinium-225 (^{225}Ac) is particularly attractive due to its suitable half-life (9.9 days) and emission of multiple alpha particles, enabling effective tumor cell killing even in micrometastatic disease. Two clinically relevant ^{225}Ac-labeled radiopharmaceuticals are **[^{225}Ac]Ac-DOTA-TATE**, targeting somatostatin receptors in neuroendocrine tumors, and **[^{225}Ac]Ac-PSMA-D4**, directed against the prostate-specific membrane antigen (PSMA) in prostate cancer.

Given the high energy and radiotoxicity of alpha emitters, strict quality control (QC) is essential to ensure patient safety, therapeutic efficacy, and regulatory compliance. QC procedures must confirm radiochemical purity, stability, identity, and sterility of the final products before clinical use. Even minor deviations in labeling efficiency or chemical composition can significantly impact biodistribution, dosimetry, and toxicity profiles.

This study focuses on the development of quality control protocols for [^{225}Ac]Ac-DOTA-TATE and [^{225}Ac]Ac-PSMA-D4, emphasizing their critical role in the safe and reproducible clinical application of targeted alpha therapy.

AIM OF THE STUDY

The aim of this study was to develop a reliable HPLC and TLC methods for the quality control of [^{225}Ac]Ac-DOTA-TATE and [^{225}Ac]Ac-PSMA-D4, ensuring accurate and reproducible analytical results by accounting for the radioactive equilibrium between the ^{225}Ac/^{221}Fr and ^{225}Ac/^{213}Bi decay pairs, which critically affect the measured activity distribution and radiochemical purity of the final products.

MATERIALS AND METHODS

HPLC analysis were conducted on Shimadzu Prominence HPLC system. For both PSMA-D4 and DOTA-TATE Phenomenex Kinetex C18, 150x4.6mm, 5 µm, 100Å column was used. The detailed HPLC parameters are presented in Table 1. Following chromatographic separation, eluate fractions were collected every 10 seconds into separate tubes. The samples were measured using Wallac Wizard 1470 automatic gamma counter at two time points: 30 minutes post-collection on "Fr-221" channel (180-220 keV, measurement time 120s) and 24h post-collection on "OPEN" channel (0-1024 keV, measurement time 180s).

TLC analysis were performed using glass microfiber chromatography paper impregnated with silica gel (iTLC-SG, Agilent) with 1M citrate buffer pH 5.0 as a mobile phase. Chromatography paper was cut into 11.5x2 cm strips. 5 µl of analyzed sample was applied on each strip. For each analysis, 4 four strips were developed. Two strips were cut into two parts: a 7 cm from the beginning of the strip and a remaining 3 cm. These parts were placed into test tube and measured in Wallac Wizard 1470 automatic gamma counter, following the same protocol as for HPLC fractions: 30 minutes after development on "Fr-221" channel and 24h after development on "OPEN" channel. The remaining two strips were analyzed 24h after developing using Cyclone Plus Storage Phosphor System (PerkinElmer, USA).

During the quality control method development phase, three TLC scanners were used: Cyclone Plus Storage Phosphor System (PerkinElmer, USA), Mini-Scan (Eckert&Ziegler, Germany) and Wallac 1470 (LKB, Sweden). The scanners parameters are shown in table 3. The TLC strips were measured approximately 30 minutes after development and again after 24 hours in order to select an the most accurate and repeatable method.

RESULTS

During the quality control method development phase, two TLC strips were evaluated using three different scanners, described in the previous section. The radiochemical purity (RCP) results for [^{225}Ac]Ac-PSMA-D4 are summarized in a table below.

	Measurement after ~30 min				Measurement after 24h			
	Cyclone Plus	Mini-Scan	Wallac 1470	HPLC	Cyclone Plus	Mini-Scan	Wallac 1470	HPLC
RCP	91.2% ± 0.5%	95.3% ± 1.4%	97.4% ± 0.4%	96.5%	97.7% ± 0.1%	98.2% ± 0.8%	96.8% ± 0.1%	97.3%

Tab.4 Radiochemical purity (RCP) of [^{225}Ac]Ac-PSMA-D4 determined by HPLC and TLC using three different detection systems.

The results obtained approximately 30 minutes and 24 hours after strip development show the expected increase in apparent RCP due to the establishment of radioactive equilibrium between ^{225}Ac and its short-lived daughters (^{221}Fr, ^{213}Bi). Reliable and reproducible RCP determination using **Cyclone Plus** and **Mini-Scan** systems was achieved only after 24 hours, once equilibrium had been reached. Therefore, for routine quality control purposes, the **Wallac 1470 gamma counter** was selected as the preferred method, as it enables accurate measurements at both time points.

Based on these findings, routine quality control of [^{225}Ac]Ac-PSMA-D4 was subsequently performed using the Wallac 1470 gamma counter for measuring both the HPLC collected fractions and TLC strips. The results obtained for samples prepared according to the enhanced labelling protocol are summarized in Table 5 and confirm the reproducibility and stability of the optimized analytical procedure.

	Measurement after ~30 min		Measurement after 24h	
	TLC Wallac 1470	HPLC	TLC Wallac 1470	HPLC
RCP	98.5% ± 0.2%	97.2%	98.5% ± 0.5%	98.8%

Tab.5 Radiochemical purity (RCP) of [^{225}Ac]Ac-PSMA-D4 determined by HPLC and TLC using enchanced labelling protocol

CONCLUSION

The developed HPLC and TLC methods enable accurate and reproducible quality control of [^{225}Ac]Ac-DOTA-TATE and [^{225}Ac]Ac-PSMA-D4, ensuring reliable assessment of radiochemical purity and stability. Comparative evaluation of three TLC detection systems demonstrated that precise quantification of RCP using Cyclone Plus and Mini-Scan is achievable only after radioactive equilibrium between ^{225}Ac and its short-lived daughters (^{221}Fr, ^{213}Bi) has been established.

Consequently, the Wallac 1470 gamma counter was selected as the preferred method for routine QC, as it provides consistent and accurate results within 30 minutes after analysis. The application of the enhanced labelling protocol yielded highly stable complexes with RCP values exceeding 97%, confirming the robustness of the developed analytical approach and its suitability for supporting the clinical implementation of ^{225}Ac-based alpha therapy.

HPLC

Parameter	[^{225}Ac]Ac-PSMA-D4	[^{225}Ac]Ac-DOTA-TATE
Mobile phase	A: 0.1% TFA in H_2O B: 0.1% TFA in ACN Gradient elution: 0 min: 90%A 10%B 15 min: 70%A 30%B 17 min: 90%A 10%B	A: 0.1% TFA in H_2O B: 0.1% TFA in ACN Isocratic elution: 80%A 20%B
Flow	1.0 mL/min	1.0 mL/min
Column oven	40 °C	40 °C
Analysis time	17 min	15 min

Tab.1 HPLC parameters for determination of radiochemical yield

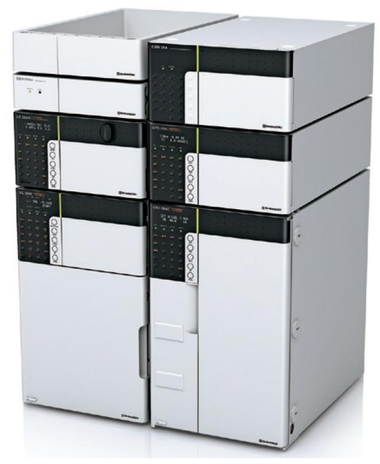

Fig 1. Shimadzu Prominence HPLC System

TLC

TLC parameters	
Mobile phase	0.1M citrate buffer pH 5.0
TLC plates	Agilent iTLC-SG paper, 11.5 x 2 cm

Tab.2 TLC plates parameters

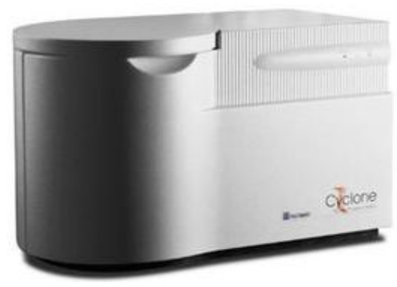

Fig 2. PerkinElmer Cyclone Plus Storage Phosphor System

TLC equipment	
Cyclone Plus Storage Phosphor System	Developed TLC strip exposure time: 5 min.
Mini-Scan	Plate movement speed: 1 mm/s PMT detector
Wallac 1470 automatic gamma counter	„Fr-221" channel: 180-220 keV „OPEN" channel: 0-1024 keV

Tab.3 TLC equipment parameters