

Evaluation of the Radiochemical Stability of ^{90}Y -DOTA-TATE with Antioxidant Stabilizers

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1. Introduction

Yttrium-90 (^{90}Y) is a β^- -emitting radionuclide that can be used for peptide receptor radionuclide therapy (PRRT) after radiolabeling with DOTA-conjugated peptides such as DOTA-TATE. For therapeutic radiopharmaceuticals containing relatively high radioactivity, maintaining radiochemical stability after radiolabeling is important because radiolytic degradation can decrease the fraction of intact radio labeled compound. Radical scavengers such as gentisic acid and ascorbic acid have been reported to suppress the radiolytic degradation of ^{90}Y -labeled DOTA conjugates [1]. In addition, radio-high-performance liquid chromatography (radio-HPLC) and radio-thin-layer chromatography (radio-TLC) are widely used to evaluate the radiochemical purity of radiolabeled DOTA peptides [2].

In this study, ^{90}Y -DOTA-TATE was prepared with and without stabilizers, and the time-dependent radiochemical stability of the labeled compound was evaluated using radio-HPLC and instant thin-layer chromatography (iTLC).

2. Methods and Results

2.1 Preparation of ^{90}Y -DOTA-TATE

A 0.4 M acetate buffer at pH 4.5 was used for the radiolabeling reaction. DOTA-TATE (250 μg) was dissolved in 1 mL of acetate buffer and reacted with ^{90}Y . The initial radioactivity was approximately 118 mCi for the non-stabilized group and 110 mCi for the stabilized group. Radiolabeling was performed at 90 $^{\circ}\text{C}$ for 30 min with gentle mixing at 10-min intervals.

For the stabilized group, ascorbic acid was added to the acetate buffer at a concentration of 0.1 mg/mL before radiolabeling. After completion of the labeling reaction and cooling to room temperature, 1 mL of stabilizer solution containing 12 mg of gentisic acid and 2 mg of ascorbic acid was additionally added.

2.2 Radio-HPLC Analysis

The radiochemical characteristics of ^{90}Y -DOTA-TATE were evaluated by reversed-phase HPLC equipped with a radioactivity detector. An Inertsil ODS-2 C18 column (4.5 $\times$ 150 mm) was used. The mobile phases consisted of water containing 0.1% (v/v) trifluoroacetic acid and

acetonitrile containing 0.1% (v/v) trifluoroacetic acid at an 80:20 ratio, with a total flow rate of 0.5 mL/min.

Cold Y-DOTA-TATE and ^{90}Y -DOTA-TATE showed characteristic peaks at approximately 26–27 min, allowing the labeled compound to be identified. The stability of ^{90}Y -DOTA-TATE was subsequently evaluated at 2, 4, 24, and 144 h after radiolabeling.

Immediately after radiolabeling, no distinct difference was observed between the stabilized and non-stabilized groups. However, in the non-stabilized group, the main ^{90}Y -DOTA-TATE peak at approximately 27 min markedly decreased after 2 h, accompanied by the appearance of additional peaks at approximately 3, 5, and 13 min. After 24 h, the main ^{90}Y -DOTA-TATE peak was no longer clearly observed and the chromatogram was dominated by degradation-related peaks.

In contrast, the stabilized group showed a slower decrease in the ^{90}Y -DOTA-TATE peak and slower formation of additional peaks with increasing storage time. These results indicate that the combination of gentisic acid and ascorbic acid delayed the time-dependent degradation of ^{90}Y -DOTA-TATE.

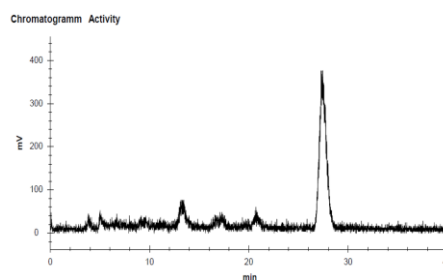


Fig. 1. HPLC chromatogram of ^{90}Y -DOTA-TATE without stabilizers.

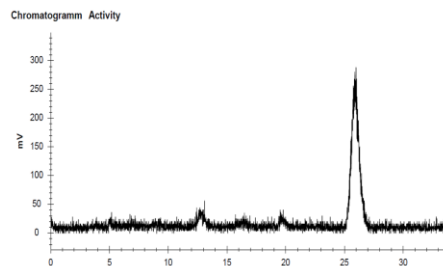


Fig. 2. HPLC chromatogram of ^{90}Y -DOTA-TATE with stabilizers.

2.3 iTLC Analysis

The radiochemical stability was additionally evaluated by iTLC using two developing systems, including 75% methanol and a water/ethanol/HCl-based mobile phase. The labeled ^{90}Y -DOTA-TATE and unbound ^{90}Y species could be distinguished according to their different migration behavior. The iTLC results showed a rapid decrease in the fraction corresponding to intact radiolabeled compound with increasing time in the non-stabilized group. In contrast, the stabilized group maintained a higher fraction of labeled compound during the early observation period. However, substantial degradation was also observed in the stabilized sample after 144 h. The trends observed by iTLC were consistent with those obtained from radio-HPLC, supporting the protective effect of the stabilizer formulation on ^{90}Y -DOTA-TATE.

Table 1. Time-dependent iTLC scan result of ^{90}Y -DOTA-TATE using methanol 75% as the mobile phase.

	With stabilizers		Without stabilizers	
	^{90}Y -DOTA-TATE ROI	Free ^{90}Y ROI	^{90}Y -DOTA-TATE ROI	Free ^{90}Y ROI
0 h	95.89%	4.11%	90.22%	9.78%
2 h	93.45%	6.55%	71.59%	28.41%
4 h	94.57%	5.43%	65.49%	34.51%
24 h	87.08%	12.92%	58.86%	43.14%
144 h	28.43%	71.57%	28.14%	71.83%

Table 2. Time-dependent iTLC scan result of ^{90}Y -DOTA-TATE using water/ethanol/HCl as the mobile phase.

	With stabilizers		Without stabilizers	
	^{90}Y -DOTA-TATE ROI	Free ^{90}Y ROI	^{90}Y -DOTA-TATE ROI	Free ^{90}Y ROI
0 h	94.87%	5.13%	92.05%	7.95%
2 h	93.59%	6.41%	68.59%	31.41%
4 h	93.57%	6.43%	57.81%	42.19%
24 h	67.09%	32.91%	47.58%	52.42%
144 h	30.81%	69.19%	9.43%	90.57%

3. Conclusions

The time-dependent radiochemical stability of ^{90}Y -DOTA-TATE was evaluated in the presence and absence of gentisic acid and ascorbic acid. In the absence of stabilizers, the characteristic ^{90}Y -DOTA-TATE peak rapidly decreased and degradation-related peaks increased within several hours after radiolabeling. Addition of gentisic acid and ascorbic acid delayed the decrease of the intact ^{90}Y -DOTA-TATE fraction, and the same tendency was observed by both radio-HPLC and iTLC. These results suggest that the combination of gentisic acid and ascorbic acid can improve the short-

term radiochemical stability of ^{90}Y -DOTA-TATE under the investigated labeling conditions. Further quantitative evaluation of radiochemical purity and optimization of stabilizer concentration would be useful for establishing an appropriate formulation for high-activity ^{90}Y -DOTA-TATE.

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