

## <sup>99m</sup>Tc-exendin-4: Radiolabeling and quality control studies of glucagon-like peptide analog

Derya Ilem Ozdemir,  Meliha Ekinci,  Emre Ozgenc,  Evren Atlıhan Gundogdu,   
Makbule Asikoglu 

Department of Radiopharmacy, Ege University, Faculty of Pharmacy, Izmir, Türkiye

### ABSTRACT

**Aim:** Exendin-4 is a type 2 diabetes antidiabetic drug that is a peptide agonist of the glucagon-like peptide 1 receptor (GLP-1R). Up to date, different exendin-4 compounds have been radiolabeled with many radioisotopes such as <sup>68</sup>Ga, <sup>18</sup>F, <sup>64</sup>Cu and <sup>99m</sup>Tc for various purposes like study of over expression of GLP-1R in insulinoma. The purpose of this study is to radiolabel exendin-4 with <sup>99m</sup>Tc under appropriate conditions.

**Methods:** In this study, exendin-4 was labeled with <sup>99m</sup>Tc, and quality control tests of <sup>99m</sup>Tc-exendin-4 were done using ascending radioactive thin layer chromatography (RTLC). Also, the effects of important parameters such as the amount of reducing agent, pH value, incubation time, and radiation dose on the labeling efficiency were investigated. Then, the stability of <sup>99m</sup>Tc-exendin-4 was assessed.

**Results:** According to the results, <sup>99m</sup>Tc-exendin-4 was prepared with over 95 percent labeling efficiency by a novel, easy, and quick direct method with 30-min incubation time at pH 6.6. To achieve the best radiolabeling condition; 10 µg of exendin-4, 50 µg of stannous chloride (reducing agent) and 37 MBq <sup>99m</sup>Tc was used. The RTLC studies indicated that <sup>99m</sup>Tc-exendin-4 is stable up to 6 h in room temperature.

**Conclusion:** The obtained results demonstrated that radiolabeled exendin-4 may be a promising agent for GLP-1R imaging studies. Further studies are in progress in order to evaluate receptor binding capacity and biodistribution of the complex in experimental animals.

**Key words:** Exendin-4, technetium-99m, radiolabeling, radiopharmaceuticals.

✉ Derya Ilem Ozdemir,

Department of Radiopharmacy, Ege University, Faculty of Pharmacy, Izmir, Türkiye

E-mail: [deryailem@gmail.com](mailto:deryailem@gmail.com)

Received: 2022-03-28 / Revisions: 2022-04-18

Accepted: 2022-05-05 / Published: 2022-07-01

### Introduction

Peptide receptor targeting of cancer cells with radiopharmaceuticals has recently become a popular strategy for cancer diagnosis and therapy. The glucagon-like peptide-1 receptor (GLP-1R) is a protein located on beta cells in the pancreas, as well as neurons in the brain, vagal neurons in the stomach and intestines, and

the kidney [1-4]. Drugs known as GLP-1R agonists have been developed to treat diabetes mellitus using GLP-1R as a target [5]. GLP-1R agonists with low specific activity and high renal uptake are the main clinical tracers available [6].

Exendin-4, a 39-amino-acid peptide isolated in the venom of the lizard *Heloderma suspectum* (Gila monster), is a GLP-1R agonist [7]. It has been approved for clinical use as an antidiabetic drug for the treatment of type 2 diabetes by the Food and Drug Administration, and it has been found to be safe and effective in people [8]. A small number of clinical investigations were

conducted after several exendin-4 derivatives were radiolabeled with different radionuclides ( $^{111}\text{In}$ ,  $^{123}\text{I}$ ,  $^{18}\text{F}$ ,  $^{68}\text{Ga}$ ,  $^{64}\text{Cu}$ ,  $^{89}\text{Zr}$  and  $^{99\text{m}}\text{Tc}$ ) and analyzed for nuclear imaging [9-20]. Furthermore, some researchers mentioned that when radiolabeled exendin-4 and its derivatives are administered to the body, they have significant levels in the pancreas and kidney [21,22].

There are radioactive and pharmaceutical components in radiopharmaceuticals. It is important considering factors such as the radiation dose, cost, and availability when selecting an appropriate radionuclide for radiolabeling experiments. Technetium-99m ( $^{99\text{m}}\text{Tc}$ ) has recently become the most preferred radionuclide for labeling research [23]. The use of  $^{99\text{m}}\text{Tc}$  may improve image quality and radiation safety for patients and personnel due to many procedural advantages related to this isotope's physical features [24]. Because  $^{99\text{m}}\text{Tc}$  is a radionuclide with monoenergetic gamma rays of 140 KeV, a half-life of 6 h, and a diverse chemistry for forming complexes [25].

The purpose of this study is to develop a novel radiopharmaceutical that can be targeted for GLP-1R. For this aim, exendin-4 was labeled with  $^{99\text{m}}\text{Tc}$  by appropriate conditions. Labeling efficiency and *in vitro* stability of  $^{99\text{m}}\text{Tc}$ -exendin-4 were investigated *via* radioactive thin layer chromatography (RTLC) studies in the framework of pre-study.

### Materials and methods

**Materials:** Astra-Zeneca (Turkey) provided exendin-4 as a pharmaceutical part of radiopharmaceutical. Merck (Germany) provided stannous chloride, which was employed as a reducing agent in labeling experiments. The  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  generator yielded  $^{99\text{m}}\text{TcO}_4^-$  (Nuclear Medicine Department of Ege University). Merck (Germany) provided all of

the solvents. All of the chemicals and solvents used were analytical grade and did not require further purification. A counting device (Atomlab 100 Dose Calibrator Biodex Medical Systems) and a TLC scanner (BioScan AR 2000) were used to count radioactive samples.

**Radiolabeling studies:** Different amounts of reducing agent, different radioactivity doses, pH values and incubation times were used to test the radiolabeling of  $^{99\text{m}}\text{Tc}$ -exendin-4. The radiochemical purity (RP) of the samples was measured using radio thin layer chromatography (RTLC) [26-28].

**Effect of reducing agent amount:** The reducing agent in this experiment was stannous chloride. 10  $\mu\text{g}$  of exendin-4 was dissolved in 1 mL 0.9 percent sodium chloride solution. Under the influence of a bubbling nitrogen environment, reducing agent was added to the stock solution.  $^{99\text{m}}\text{Tc}$  was reduced with different quantities of stannous chloride (25, 50, 75, and 100  $\mu\text{g}$ ) at neutral pH (1 mg reducing agent diluted in 1 mL 0.9 percent sodium chloride solution). Before the radiochemical analysis, 37 MBq (1 mCi)  $^{99\text{m}}\text{Tc}$  was radiolabeled in 0.1 mL 0.9 percent sodium chloride solution and left to remain at room temperature during 30 min.

**Effect of pH value:** The effect of pH value on  $^{99\text{m}}\text{Tc}$ -exendin-4 labeling efficiency was investigated for pH 5.6 to 8.3. For this purpose, the pH value of  $^{99\text{m}}\text{Tc}$ -exendin-4 was adjusted to 5.6, 6.6, 7.4 and 8.3 after labeling using 0.01 N HCl and 0.01 N NaOH solutions. Then, the labeling stability of the compounds which has different pH value was evaluated by RTLC studies every hour.

**Effect of incubation time:** To determine the best incubation time of  $^{99\text{m}}\text{Tc}$ -exendin-4, RTLC experiments were performed to determine the complex's RP at 5-, 15-, 30-, 45- and 60-min after labeling.

**Effect of radioactivity doses:** *In vitro* labeling investigations were carried out with 37 MBq  $^{99m}\text{Tc}$  because of the radiation safety of the employees and the environment [29]. Because higher radiation doses are used in human experiments with radiopharmaceuticals, the RP of  $^{99m}\text{Tc}$ -exendin-4 was tested with both 37 and 370 MBq radiation doses.

**Quality control studies:** Quality control of  $^{99m}\text{Tc}$ -exendin-4 were performed *via* RTLC studies. For this purpose, free  $^{99m}\text{Tc}$  was determined using Whatman 3MM chromatographic papers as the stationary phase and 100% acetone as the mobile phase. Instant thin layer chromatography-silica gel coated plates (ITLC-SG) were used to determine R/H  $^{99m}\text{Tc}$  in a solvent system of Acetonitrile/Water/Trifluoroacetic acid (ACN/W/TFA; 50/25/1.5). The radioactivity of the stationary phases was assessed using a TLC scanner, and the RP of  $^{99m}\text{Tc}$  was estimated using following equation [30,31]:

$$\text{RP (percent)} = 100 - [\text{Free } ^{99m}\text{Tc (percent)} + \text{R/H } ^{99m}\text{Tc (percent)}]$$

***In vitro* stability:** To test the stability of newly developed radiopharmaceutical, 0.1 mL of  $^{99m}\text{Tc}$ -exendin-4 and 0.4 mL of 0.9 percent sodium chloride solution was mixed [32]. After that, the mixture was allowed to leave at room temperature for 6 h. Every hour, RTLC experiments were used to assess the complex's labeling stability.

#### **Statistical analysis**

The means and standard deviations were calculated using Microsoft Excel. The statistical significance was determined using the t test. At the 95 percent confidence level ( $p > 0.05$ ), differences were determined significant. Unless otherwise noted, all experiments were carried out in triplicate. The mean and standard error of the results are given.

## **Results and Discussion**

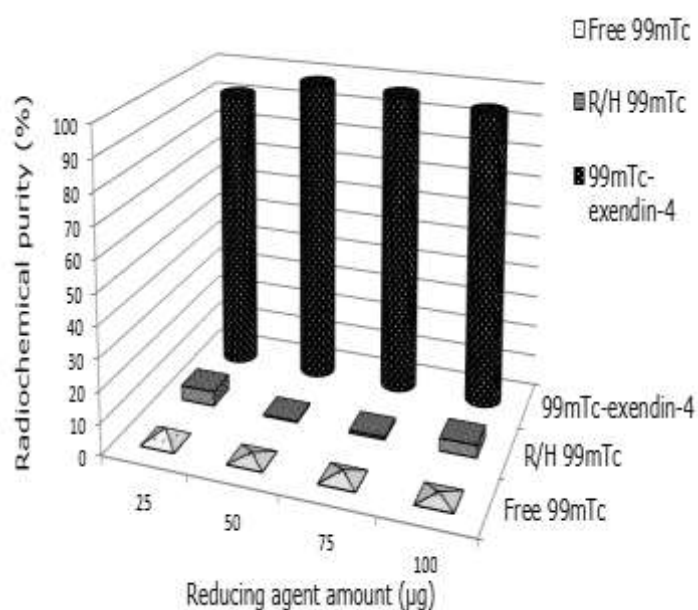
**Radiolabeling studies:** Our research group developed a novel, simple, quick, and effective direct approach for labeling of exendin-4 with  $^{99m}\text{Tc}$ . The effect of reducing agent amount, pH value, incubation time and radiation doses on radiolabeling of  $^{99m}\text{Tc}$ -exendin-4 was evaluated using RTLC technique [26-28].

In clinical trials, an exendin-4-derived compound was first labeled using In-111 ([Lys40(Ahx-DOTA- $^{111}\text{In}$ )NH<sub>2</sub>]-exendin-4) and showed high uptake in insulinomas [14]. However, because  $^{111}\text{In}$  is costly and carries a relatively high radiation damage for the patient, researchers evaluated tumor uptake *in vivo* by labeling the same compound with  $^{68}\text{Ga}$  and  $^{99m}\text{Tc}$ .

[Lys40(Ahx-HYNIC- $^{99m}\text{Tc}$ /EDDA)NH<sub>2</sub>]-exendin-4, compared with ([Lys40(Ahx-DOTA- $^{111}\text{In}$ )NH<sub>2</sub>]-exendin-4 and Lys40(Ahx-DOTA- $^{68}\text{Ga}$ )NH<sub>2</sub>]-exendin-4, showed significantly less tumor and organ uptake, but did not result reduced image quality [22]. Based on these promising results of exendin-4 derivative compound in insulinoma imaging, we performed radiolabeling and quality control studies with  $^{99m}\text{Tc}$  for the usability of exendin-4 as a GLP-1R imaging agent without long labeling process, heating, boiling, and purification.

**Effect of reducing agent amount:** In the  $^{99m}\text{Tc}$  (VII) oxidation state,  $^{99m}\text{TcO}_4^-$  was eluted from a  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator. For  $^{99m}\text{TcO}_4^-$ , this level is unable to label directly with any component. So, before labeling,  $^{99m}\text{TcO}_4^-$  is reduced to transform it from a +7-oxidation state to a +4/+5-oxidation state, which allows it to form complexes with the ligand and synthesize the radiopharmaceutical [33]. Various reducing agents have been utilized for this purpose, with stannous chloride being one of the most common [23,24].

Radiolabeling tests were carried out to see how the amount of reducing agent affected the labeling yield. First, the labeling efficiency was increased by increasing the amount of stannous chloride. When the reducing agent concentration was increased above the optimum value, the labeling efficiency decreased slightly, but was not significantly ( $p>0.05$ ) (Figure 1).



**Figure 1.** Variation of free  $^{99m}\text{Tc}$ , R/H  $^{99m}\text{Tc}$  and  $^{99m}\text{Tc}$ -exendin-4 as a function of different stannous chloride amount.

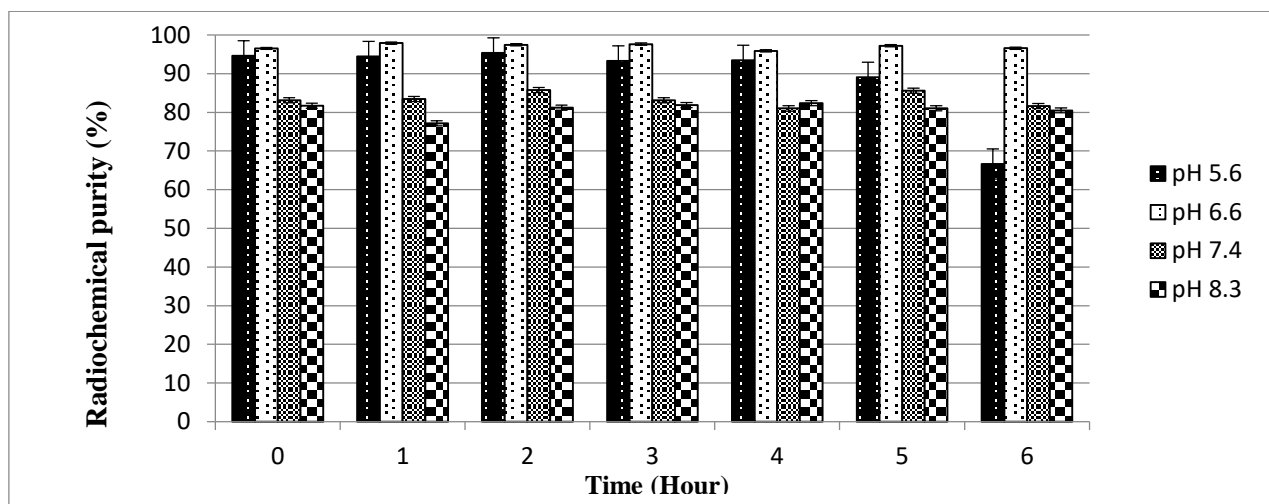
The RP (percent) of various concentrations of stannous chlorides is shown in Table 1. The amount of reducing agent that was shown to be the most effective was 50  $\mu\text{g}$ , according to the findings. Labeling efficiency was above 95 percent under these settings and did not alter much after 6 h at room temperature ( $p>0.05$ ).

**Effect of pH:** Although radiopharmaceuticals were required to modify the body pH, small-volume preparations were pH stable [32]. For pH 5.6-8.3, the effect of pH value on  $^{99m}\text{Tc}$ -exendin-4 labeling efficiency was investigated. According to the results of the experiments, the pH of the reaction medium plays a crucial role in the labeling process (Figure 2). Labeling efficiency significantly changed while keeping other reaction parameters constant and varying the pH of the reaction from 5.6 to 8.3 ( $p<0.05$ ). At pH 6.6, maximum labeling efficiency was observed, and  $^{99m}\text{Tc}$ -exendin-4 was found stable up to 6 h ( $p>0.05$ ).

**Effect of incubation time:** To determine the best incubation time, firstly, 50  $\mu\text{g}$  stannous chlorides including compounds were labeled with  $^{99m}\text{Tc}$ . After labeling, the RP of  $^{99m}\text{Tc}$ -exendin-4 were investigated with RTLC studies which performed at determined times post-

**Table 1.** Radiochemical purity (percent) of  $^{99m}\text{Tc}$ -exendin-4 with various concentrations of stannous chlorides.

Radiochemical purity (%)				
Time (h)	Stannous chloride ( $\mu\text{g}$ )			
	25	50	75	100
0.5	89.84 $\pm$ 0.70	96.53 $\pm$ 0.82	96.68 $\pm$ 0.11	92.75 $\pm$ 0.78
1	91.51 $\pm$ 1.33	97.91 $\pm$ 0.76	96.94 $\pm$ 0.52	94.35 $\pm$ 0.49
2	92.34 $\pm$ 1.35	97.48 $\pm$ 0.72	92.91 $\pm$ 1.72	92.86 $\pm$ 1.27
3	89.58 $\pm$ 2.53	97.64 $\pm$ 1.10	96.56 $\pm$ 0.99	94.09 $\pm$ 0.66
4	80.69 $\pm$ 0.84	95.90 $\pm$ 0.51	93.15 $\pm$ 0.48	92.30 $\pm$ 1.12
5	85.72 $\pm$ 2.90	97.24 $\pm$ 1.62	93.99 $\pm$ 0.75	90.75 $\pm$ 0.83
6	83.81 $\pm$ 1.45	96.60 $\pm$ 1.00	93.79 $\pm$ 0.57	93.79 $\pm$ 0.83



**Figure 2:** The effect of the pH on labeling efficiency of <sup>99m</sup>Tc-exendin-4.

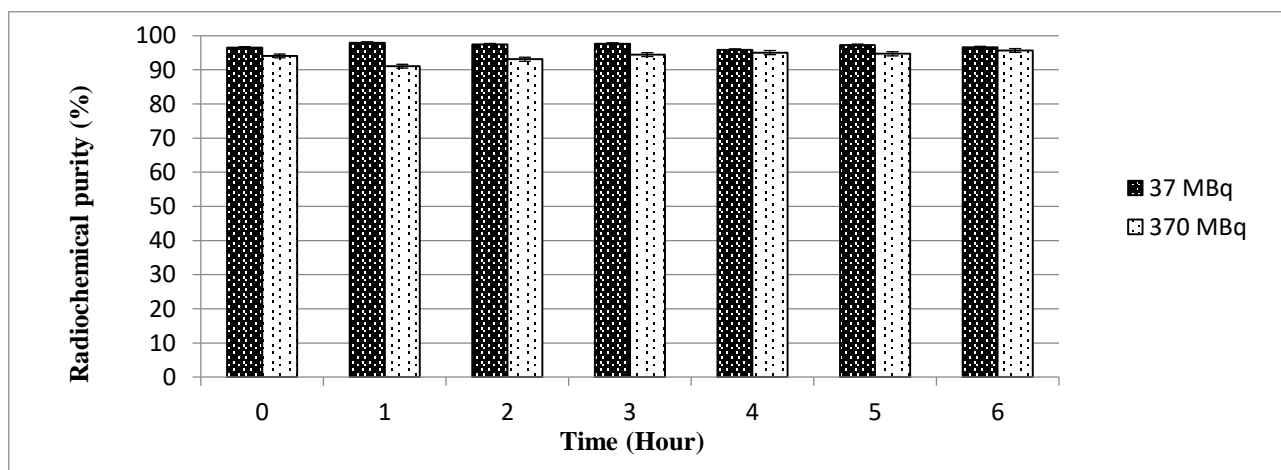
labeling (Table 2). According to results, the RP of <sup>99m</sup>Tc-exendin-4 was reached over 95% in 30 min after labeling. The best radiolabeling yield (~95%) was observed after a 30-min incubation period, while prolonged incubation intervals had no noticeable effect ( $p > 0.05$ ). This result shows that <sup>99m</sup>Tc-exendin-4 can be used in nuclear medicine after 30 min.

**Table 2.** The effect of incubation time on radiochemical purity.

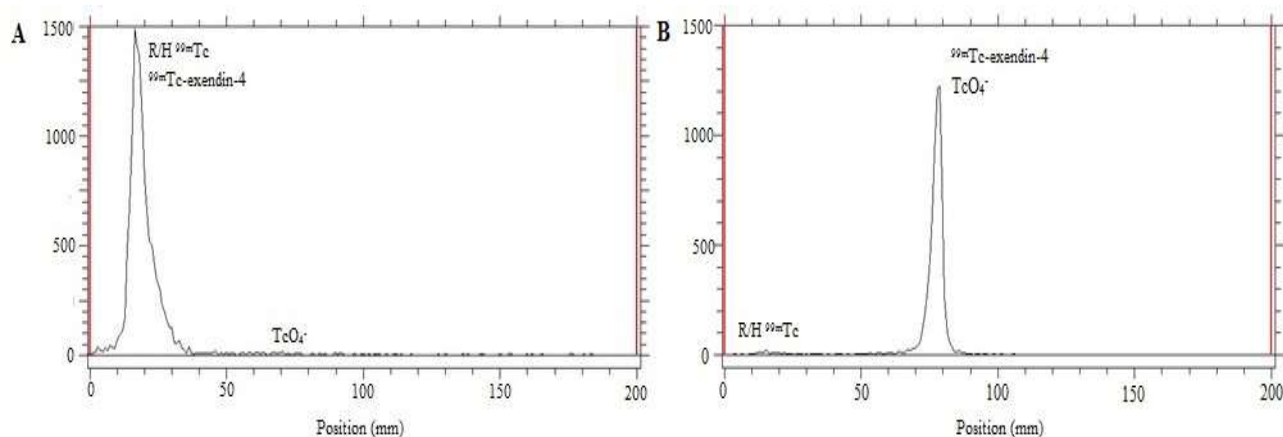
Time (min)	Radiochemical purity (%)
5	88.82 ± 2.66
15	89.24 ± 2.16
30	96.93 ± 0.26
45	93.43 ± 0.54
60	94.72 ± 0.44

**Effect of radioactivity doses:** Exendin-4 was labeled with 37 and 370 MBq <sup>99m</sup>Tc radiation doses. Slightly decrease in RP of <sup>99m</sup>Tc-exendin-4 complex was observed with increasing of radioactivity (Figure 3) ( $p > 0.05$ ). As a result of increasing the amount of radioactivity 10 folds, the RP value was found over 90% ( $p > 0.05$ ). So, <sup>99m</sup>Tc-exendin-4 has been found suitable for labeling with high radioactivity in nuclear medicine.

**Quality control studies:** For quality control of radiopharmaceuticals, high-performance liquid chromatography (HPLC), RTLC and/or gas chromatography (GC) can be used [34]. Because of fast and safe, in this study, RTLC technique were used to evaluate of the labeling efficiency of <sup>99m</sup>Tc-exendin-4. To



**Figure 3.** The effect of radioactivity doses (37 and 370 MBq <sup>99m</sup>Tc) on radiochemical purity.



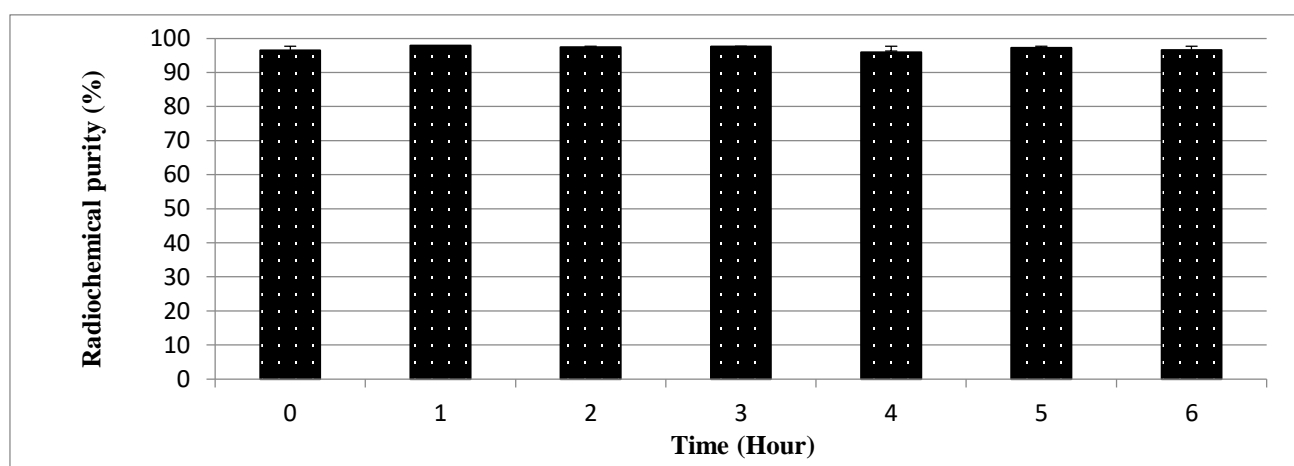
**Figure 4.** RTLC chromatogram of  $^{99m}\text{Tc}$ -exendin-4 in different mobile phases: A: Acetone, B: ACN/W/TFA (50/25/1.5).

identify and measure the levels of radioactive impurities (Free  $^{99m}\text{Tc}$ , R/H  $^{99m}\text{Tc}$ ), two solvent systems were utilized. Free  $^{99m}\text{Tc}$  migrated with the solvent front in acetone, whereas  $^{99m}\text{Tc}$ -exendin-4 and R/H  $^{99m}\text{Tc}$  remained at the spotting point. The mobile phase ACN/W/TFA (50/25/1.5) was used to identify R/H  $^{99m}\text{Tc}$ , with R/H  $^{99m}\text{Tc}$  remaining at the point of spotting while free  $^{99m}\text{Tc}$  and  $^{99m}\text{Tc}$ -exendin-4 migrated with the solvent front. The RTLC chromatogram of  $^{99m}\text{Tc}$ -exendin-4 was presented in Figure 4. The RP of  $^{99m}\text{Tc}$ -exendin-4 was over 95 percent, acquired *via* RTLC.

patients in nuclear medicine after they have been dissolved in 0.9 percent sodium chloride solution and labeled with  $^{99m}\text{Tc}$ . For this reason, the stability of the developed radiopharmaceutical was checked up to 6 h at room temperature. During the incubation period,  $^{99m}\text{Tc}$ -exendin-4 was found stable in 0.9 percent sodium chloride solution with high labeling efficiency ( $p > 0.05$ ) (Figure 5).

### Conclusion

In this study, we demonstrated that exendin-4 can be labeled with  $^{99m}\text{Tc}$  with a high labeling efficiency (>95%) using a simple RTLC



**Figure 5.** The stability of  $^{99m}\text{Tc}$ -exendin-4 in saline up to 6 hours.

**In vitro stability:** Cold kits (consisting of pharmaceutical part, reducing agent and/or antioxidant agent) are usually applied to

technique. The produced complex was extremely stable, with labeling efficiency continuing up to 6 h. With 50  $\mu\text{g}$  stannous

chloride and 37 MBq  $^{99m}\text{TcO}_4^-$  containing formulations at pH 6.6 at room temperature, the highest RP was achieved. Also, further studies with  $^{99m}\text{Tc}$ -exendin-4 are in progress in order to evaluate receptor binding capacity and biodistribution and imaging of the complex in experimental animals.

**Acknowledgement:** *The authors also thank the Nuclear Medicine Department at Ege University for providing the  $^{99m}\text{TcO}_4^-$ .*

**Funding:** *TLC Scanner was funded by T.R. Prime Ministry State Planning Organization Foundation Grant Project Number: 09DPT001, which the authors gratefully appreciate.*

**Conflict of Interest:** *The authors declare that they have no conflict of interest.*

**Ethical Statement:** *Ethics committee decision was not taken as this research was a laboratory study.*

### **Open Access Statement**

*Experimental Biomedical Research is an open access journal and all content is freely available without charge to the user or his/her institution. This journal is licensed under a [Creative Commons Attribution 4.0 International License](#). Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.*

**Copyright (c) 2021:** Author (s).

### **References**

- [1]Thorens B. Expression cloning of the pancreatic beta cell receptor for the glucagon-like peptide 1. Proc Natl Acad Sci USA. 1992; 89(18): 8641–45.
- [2]Dillon JS, Tanizawa Y, Wheeler MB, et al. Cloning and functional expression of the human glucagon-like peptide-1 (GLP-1) receptor. Endocrinology. 1993; 133(4): 1907–10.
- [3]Cork SC, Richards JE, Holt MK, et al. Distribution and characterisation of Glucagon-like peptide-1 receptor expressing cells in the mouse brain. Mol Metab. 2015; 4(10): 718–31.
- [4]Williams EK, Chang RB, Strohlic DE, et al. Sensory neurons that detect stretch and nutrients in the digestive system. Cell. 2016; 166(1): 209–21.
- [5]Holst JJ. Treatment of type 2 diabetes mellitus with agonists of the GLP-1 receptor or DPP-IV inhibitors. Expert Opin Emerg Drugs. 2004; 9(1): 155–66.
- [6]Läppchen T, Tönnemann R, Eersels J, et al. Radioiodinated exendin-4 is superior to the radiometal-labelled Glucagon-Like Peptide-1 Receptor probes overcoming their high kidney uptake. PLoS One. 2017; 12(1): e0170435.
- [7]Eng J, Kleinman WA, Singh L, et al. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. J Biol Chem. 1992; 267(11): 7402–405.
- [8]Baggio LL, Drucker DJ. Biology of Incretins: GLP-1 and GIP. Gastroenterology. 2007; 132(6): 2131–57.
- [9]Selvaraju RK, Velikyan I, Asplund V, et al. Pre-clinical evaluation of [68Ga]Ga-DO3A-VS-Cys40-Exendin-4 for imaging of insulinoma. Nucl Med Biol. 2014; 41(6): 471–76.
- [10]Sowa-Staszczak A, Pach D, Mikołajczak R, et al. Glucagon-like peptide-1 receptor imaging with [Lys40(Ahx-HYNIC-

- 99mTc/EDDA)NH<sub>2</sub>]-exendin-4 for the detection of insulinoma. *Eur J Nucl Med Mol Imaging.* 2013; 40(4): 524–31.
- [11] Kiesewetter DO, Gao H, Ma Y, et al. 18F-radiolabeled analogs of exendin-4 for PET imaging of GLP-1 in insulinoma. *Eur J Nucl Med Mol Imaging.* 2012; 39(3): 463–73.
- [12] Xu Y, Pan D, Xu Q, et al. Insulinoma imaging with glucagon-like peptide-1 receptor targeting probe 18F-FBEM-Cys39-exendin-4. *J Cancer Res Clin Oncol.* 2014; 140(9): 1479–88.
- [13] Xu Q, Zhu C, Xu Y, et al. Preliminary evaluation of [18F]AlF-NOTA-MAL-Cys39-exendin-4 in insulinoma with PET. *J Drug Target.* 2015; 23(9): 813–20.
- [14] Wild D, Behe M, Wicki A, et al. [Lys40(Ahx-DTPA-111In)NH<sub>2</sub>]exendin-4, a very promising ligand for glucagon-like peptide-1 (GLP-1) receptor targeting. *J Nucl Med* 2006; 47: 2025–2033.
- [15] Mikkola K, Yim CB, Fagerholm V, et al. <sup>64</sup>Cu- and <sup>68</sup>Ga-labelled [Nle(14),Lys(40)(Ahx-NODAGA)NH<sub>2</sub>]-exendin-4 for pancreatic beta cell imaging in rats. *Mol Imaging Biol.* 2014; 16: 255–63.
- [16] Wu Z, Liu S, Nair I, et al. (<sup>64</sup>Cu) labeled sarcophagine exendin-4 for microPET imaging of glucagon like peptide-1 receptor expression. *Theranostics.* 2014; 4: 770–77.
- [17] Bauman A, Valverde IE, Fischer CA, et al. Development of <sup>68</sup>Ga- and <sup>89</sup>Zr-labeled exendin-4 as potential radiotracers for the imaging of insulinomas by PET. *J Nucl Med.* 2015; 56: 1569–74.
- [18] Wu Z, Liu S, Hassink M, et al. Development and evaluation of 18F-TTCO-Cys40-Exendin-4: a PET probe for imaging transplanted islets. *J Nucl Med.* 2013; 54: 244–51.
- [19] Jodal A, Lankat-Buttgereit B, Brom M, et al. A comparison of three (<sup>67/68</sup>Ga)-labelled exendin-4 derivatives for beta-cell imaging on the GLP-1 receptor: the influence of the conjugation site of NODAGA as chelator. *EJNMMI Res.* 2014; 4: 31.
- [20] Gotthardt M, Fischer M, Naeher I, et al. Use of the incretin hormone glucagon-like peptide-1 (GLP-1) for the detection of insulinomas: initial experimental results. *Eur J Nucl Med Mol Imaging.* 2002; 29: 597–606.
- [21] Yim C Bin, Mikkola K, Fagerholm V, et al. Synthesis and preclinical characterization of [<sup>64</sup>Cu]NODAGA-MAL-exendin-4 with a Nε-maleoyl-l-lysyl-glycine linkage. *Nucl Med Biol.* 2013; 40(8): 1006–1012.
- [22] Wild D, Wicki A, Mansi R, et al. Exendin-4-based radiopharmaceuticals for glucagonlike peptide-1 receptor PET/CT and SPECT/CT. *J Nucl Med.* 2010; 51(7): 1059–1067.
- [23] Boschi A, Uccelli L, Martini P. A Picture of modern Tc-99m radiopharmaceuticals: production, chemistry, and applications in molecular imaging. *Appl Sci.* 2019; 9(12): 2526.
- [24] Shahzad K, Majid ASA, Khan M, et al. Recent advances in the synthesis of (99m Technetium) based radio-pharmaceuticals. *Rev Inorg Chem.* 2021; 41(3): 151-98.
- [25] Akbar, M.U., Ahmad, M.R., Shaheen, A. et al. A review on evaluation of technetium-99m labeled radiopharmaceuticals. *J Radioanal Nucl Chem.* 2016; 310: 477–93.
- [26] Maioli C, Luciniani G, Strinchini A, et al. Quality control on radiochemical purity in Technetium-99m radiopharmaceuticals labelling: three years of experience on 2280 procedures. *Acta Biomed.* 2017; 88(1): 49-56.
- [27] İlem-Özdemir D, Karavana SY, Şenyiğit ZA, et al. Radiolabeling and cell incorporation studies of gemcitabine HCl



- microspheres on bladder cancer and papilloma cell line. *J Radioanal Nucl Chem.* 2016; 310(2): 515–22.
- [28] Khan NUH, Naqvi SAR, Sohail H, et al. Technetium-99m labeled Ibuprofen: Development and biological evaluation using sterile inflammation induced animal models. *Mol Biol Rep.* 2019; 46: 3093–3100.
- [29] Stabin M. Nuclear medicine dosimetry. *Phys Med Biol.* 2006; 51: R187.
- [30] İlem-Ozdemir D, Atlihan-Gundogdu E, Ekinci M, et al. Radiolabeling and in vitro evaluation of a new 5-fluorouracil derivative with cell culture studies. *J Label Compd Radiopharm.* 2019; 62(13): 874–84.
- [31] Jeon SJ, Kim KM, Lim I, et al. Pixelated scintillator-based compact radio thin layer chromatography scanner for radiopharmaceuticals quality control. *J Instrum.* 2017; 12: T11003.
- [32] Ekinci M, Öztürk AA, Santos-Oliveira R, İlem-Özdemir D. The use of Lamivudine-loaded PLGA nanoparticles in the diagnosis of lung cancer: Preparation, characterization, radiolabeling with <sup>99m</sup>Tc and cell binding. *J Drug Deliv Sci Technol.* 2022; 69: 103139.
- [33] Ekinci M, İlem-Ozdemir D, Gundogdu E, et al. Methotrexate loaded chitosan nanoparticles: Preparation, radiolabeling and in vitro evaluation for breast cancer diagnosis. *J Drug Deliv Sci Technol.* 2015; 30(A): 107–13.
- [34] Straub, M., Leresche, M., Pilloud, C. et al. A new two-strip TLC method for the quality control of technetium-99m mercaptoacetyl-triglycine (<sup>99m</sup>Tc-MAG3). *EJNMMI Radiopharm Chem.* 2018; 3(1): 5.