Exp Biomed Res 2023; 6(1): 45-56 **DOI: 10.30714/j-ebr.2022.169**

Experimental Biomedical Research *Original article*

A preliminary study on radiolabeling and quality control of [^{99m}Tc]Tc-6-mercaptopurine to develop tumor scintigraphic agent

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ABSTRACT

Aim: Cancer is one of the most cause of deaths in worldwide. 6-mercaptopurine (6-MP) is successfully to treat leukemia. In recent years, 6-MP has remarkable properties for treating solid tumors. The aim of this study is to radiolabel 6-MP with [^{99m}Tc]Tc under appropriate conditions to develop tumor scintigraphic agent.
Methods: In this work, 6-MP was radiolabeled using [^{99m}Tc]Tc radionuclide, and quality control experiment of [^{99m}Tc]Tc-6-MP were assessed *via* radioactive thin layer chromatography (RTLC). Also, the effect of critical parameters affecting the radiolabeling efficiency (reducing and antioxidant agent, incubation time, pH value, radiation dose) was evaluated. Then, the stability and lipophilicity tests of [^{99m}Tc]Tc-6-MP was performed.
Results: According to the results, [^{99m}Tc]Tc-6-MP was prepared with over 93% labeling efficiency by a novel, easy, and quick direct method with 15-min incubation time at pH 7. To achieve the best radiolabeling condition; 0.5 mg.mL^{-1} of 6-MP solution, 250 µg of stannous tartrate (reducing agent), 0.050 mg ascorbic acid (antioxidant agent), and 37 MBq [^{99m}Tc]Tc was used. The RTLC studies indicated that [^{99m}Tc]Tc-6-MP is stable up to 6-h in room temperature. The log*P* of the [^{99m}Tc]Tc-6-MP were found to be -0.021 ± -0.001.
Conclusions: The obtained results showed that radiolabeled 6-MP may be a promising tumor diagnostic agent. Further studies are in progress in order to evaluate tumor cell binding capacity and biodistribution of the complex in experimental animals.

Key words: 6-mercaptopurine, technetium-99m, radiolabeling, radiopharmaceuticals, tumor.

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Received: 2022-07-30 / Revisisons: 2022-09-29
Accepted: 2022-10-02 / Published: 2023-01-01

Introduction

Cancer has long been one of the world's top causes of death [1,2]. Detection and effective treatment of cancer at early levels significantly reduces the cost and duration of treatment, and the risk of mortality and morbidity [3]. Nuclear medicine imaging offers non-invasive functional data at the cellular and molecular level. Abnormalities are frequently detected at very early stages by nuclear imaging techniques [4]. Therefore, it is critical to develop radiopharmaceuticals that can detect physiological changes before anatomical changes occur.

In scintigraphic imaging studies, the appropriate radionuclide is bound with the pharmaceutical part, and the radiation emitted by the radionuclide is imaged with a gamma camera following application [5]. The scintigraphic images obtained with the camera that records the gamma rays emitted from the given radioactive

compound provide information about the functions and anatomy of the systems in the body. These tests are widely used in the world, because they are safe and non-invasive [6-8].

Radioactive and pharmaceutical components are combined in radiopharmaceuticals for diagnosis or treatment of a disease [9]. When choosing a suitable radionuclide for radiolabeling experiments, it is considered to radiation dose, cost, and availability. Ideal characteristics of [^{99m}Tc]Tc include its 140 KeV pure gamma photon emission, 6-h physical half-life, low price, and ease of supply. These features makes [^{99m}Tc]Tc the most frequently used radionuclide in nuclear medicine [10].

6-mercaptopurine (6-MP) known as a purine analogue is used successfully to treat cancer, particularly leukemia [11]. Because of its excellent coordination properties resulting from its nitrogen and sulfur donor sites, which can be bound at N-1, N-3, N-7, and N-9, 6-MP has recently received a lot of attention as an antineoplastic drug. 6-MP also possesses chemotherapeutic properties. The capacity of 6-MP to convert the nitrogen donor sites into the corresponding ribosides is thought to be the basis for its activity in cancer cells [12,13]. Dorniani *et al.* [14] developed 6-MP-coated magnetite nanoparticles which can be as an alternative drug delivery system for breast cancer treatment [14]. Also, Nezhad-Mokhtari *et al.* [15] designed polymeric nanogels including 6-MP and methotrexate for treatment of breast cancer. These smart nanogels found as effective cancer therapy agent [15]. The aim of this study is to develop a novel radiopharmaceutical to detected for tumor. For this aim, 6-MP was labeled with [^{99m}Tc]Tc by appropriate conditions. Labeling efficiency and *in vitro* stability of [^{99m}Tc]Tc-6-MP were investigated *via* radioactive thin layer chromatography (RTLC) studies in the framework of pre-study.

Materials and metods

Materials: Aspen (Feucht, Germany) provided 6-MP. Merck (Germany) provided stannous chloride, stannous tartrate, and ascorbic acid. The [^{99}Mo]Mo/[^{99m}Tc]Tc generator yielded [^{99m}Tc]$NaTcO_4$ (Nuclear Medicine Department of Ege University, Türkiye). All solvents used were of analytical grade and obtained from Merck (Germany).

Radiolabeling of [^{99m}Tc]Tc-6-MP: To determine the optimum labeling procedures, [^{99m}Tc]Tc-6-MP was evaluated in different types and quantities of reducing and antioxidant agents. Also, the effect of pH value, incubation time, radiation dose, *in vitro* stability in 0.9% sodium chloride solution (SF) on radiochemical purity (RP), and partition coefficient value were investigated. RP was measured with the help of RTLC analysis [16,17].

Effect of reducing agent type and amount on radiolabeling of [^{99m}Tc]Tc-6-MP: Stannous salts (chloride and tartrate) were used as reducing agent, individually. Firstly, 6-MP (0.5 mg) was dissolved in SF solution (1 mL). Then, stannous chloride was added to the system. The reduction of [^{99m}Tc]Tc occurred at an acidic pH (1 mg stannous chloride dissolved in 1 mL 0.01 N HCl) with different concentration of stannous chloride (10, 25, 50, 100 and 250 $\mu g.\mu L^{-1}$). Radiolabeling of 6-MP was performed with 37 MBq of [^{99m}Tc]Tc in SF (0.1 mL) and the system was incubated for 15-min.

In radiolabeling studies, stannous tartrate was also used as a reducing agent. Briefly, 6-MP (0.5 mg) was dissolved in SF (1 mL). Then, 10, 25, 50, 100 and 250 $\mu g.\mu L^{-1}$ stannous tartrate (1 mg stannous tartrate diluted in 1 mL 0.01 N HCl) and 37 MBq [^{99m}Tc]Tc was added to the solution. Prior to radiochemical analysis, the radiolabeled complexes were allowed to remain for 15-min incubation time.

Effect of antioxidant agent on radiolabeling of [^{99m}Tc]Tc-6-MP: Radiolabeling tests were conducted in the absence and presence of an antioxidant agent to assess the influence of the antioxidant agent on the radiolabeling efficiency and stability of [^{99m}Tc]Tc-6-MP. The antioxidant agent was ascorbic acid, while the reducing agent was stannous tartrate. In brief, 6-MP (0.5 mg) was dissolved in SF (1 mL). Each solution received 50, 100 and 250 µg of stannous tartrate. Radiolabeling was performed in the absence and presence of ascorbic acid (0.025 and 0.050 mg, respectively). Freshly eluted 37 MBq [^{99m}Tc]Tc was added to system, and the vials were incubated for 15-min. RTLC was used to determine the labeling efficiency.

Effect of pH on radiolabeling of [^{99m}Tc]Tc-6-MP: The effect of pH value on [^{99m}Tc]Tc-6-MP labeling efficiency was investigated for pH 2.0 to 9.0. For this purpose, the pH value of [^{99m}Tc]Tc-6-MP was adjusted to 2.0, 7.0 and 9.0 after labeling using 0.1 N HCl and 0.01 N NaOH solutions. Then, the labeling stability of the compounds which have different pH value was evaluated every hour.

Effect of incubation time on radiolabeling of [^{99m}Tc]Tc-6-MP: In order to evaluate the effect of time upon the stability of radiolabeling, the RTLC studies were performed in varied times: 5, 15, 30, 45- and 60-min after post-labeling.

Effect of dose amount on radiolabeling of [^{99m}Tc]Tc-6-MP: The radiolabeling assay has been performed at: 37, 185 and 370 MBq for the effect of the amount of [^{99m}Tc]Tc on the radiolabeling.

In vitro stability of [^{99m}Tc]Tc-6-MP: For *in vitro* stability, [^{99m}Tc]Tc-6-MP (0.1 mL) reaction media was added to SF (0.4 mL). The mixture was incubated for 6-h at room temperature and RTLC assays were performed every hour up to 6-h.

Quality control of [^{99m}Tc]Tc-6-MP: As stationary phases, Whatman 3MM plates and ITLC-SG papers were chosen. Whatman 3MM plates were used as the stationary phase and acetone (100%) as the mobile phase to measure free-[^{99m}Tc]Tc. ITLC-SG papers developed in a Acetonitrile/ Water/Trifluoroacetic acid (ACN/W/TFA; 50/25/1.5) solvent mixture was used to evaluate Reduced/Hydrolized (R/H)-[^{99m}Tc]Tc. The radioactivity on plates was assessed using a TLC scanner (Bioscan AR 2000) after chromatographic separation, and the RP (%) of [^{99m}Tc]Tc was estimated using the following equation (Eq. 1) [18,19]:

RP (%) = 100 – (Free-[^{99m}Tc]Tc (%) + R/H-[^{99m}Tc]Tc (%)) (Eq.1)

Partition coefficient study of [^{99m}Tc]Tc-6-MP: For partition coefficient study of [^{99m}Tc]Tc-6-MP, n-octanol and PBS (pH: 7) were used. In a centrifuge eppendorf, n-octanol (300 µL), PBS (pH: 7; 300 µL) and [^{99m}Tc]Tc-6-MP (150 µL) were added, mixed for 1-min, then centrifuged at 1420 x *g* for 30-min. The mixture underwent centrifugation and split into two stages. A total of 100 µL of lower and upper phase activity were counted using a gamma counter. The following equation was used to obtain the log*P* value of [^{99m}Tc]Tc-6-MP (Eq. 2) [20]:

log*P* = log (n-octanol phase/PBS phase) (Eq.2)

Statistical analysis: Using Microsoft Excel, the means and standard deviations were computed. The statistical significance was assessed using the t test. Differences that were significant at the 95% level of confidence (p>0.05) were noted.

Results and Discussion

Cancer is the leading cause of death worldwide. According to 2020 data, a total of 19.3 million new cancer cases developed in the

world, and 10.0 million people died due to cancer [2]. Cancer can be defined simply as uncontrolled cell proliferation. Although uncontrolled proliferation is the main feature, the cancer cell also has other biological characteristics. These include avoiding contact inhibition in cell cultures, not requiring external stimuli to divide, insensitivity to proliferation suppressive signals, avoiding apoptosis, stimulating angiogenesis and metastasis. Although there are many different types of cancer, they all start with the out-of-control proliferation of abnormal cells. If left untreated, it can cause serious illness and even death [21,22].

Anatomical imaging techniques are not sufficient for imaging in the initial stage of cancer, as they rely on morphological changes. Since scintigraphic imaging is a non-invasive imaging technique based on the detection of physiological changes, it allows diagnosis at an early stage [23,24]. With nuclear medicine imaging techniques, both the diagnosis of the disease and its prevalence, and in other words, at what stage is determined [25].

Radiolabeling of [^{99m}Tc]Tc-6-MP: In this study, a new, simple, quick, and efficient direct labeling approach for [^{99m}Tc]Tc-6-MP was developed. The RTLC tests were used to evaluate the RP of the radiolabeled compound.

In the past years, 6-MP was radiolabeled with different radionuclides. 6-MP was labeled with [^{14}C]C and [^{35}S]S by Adamson *et al.* [26] and [^{67}Ga]Ga by Guarino *et al.* [27] for different purposes. Hunt *et al.* [28] radiolabeled 6-MP with [^{99m}Tc]Tc for cholescintigraphy. According to the study, the [^{99m}Tc]Tc-6-MP was cleared rapidly from the blood and concentrated in the bile. So, the researchers concluded that this finding prompted further assessment of the compound as a radiopharmaceutical for cholescintigraphy [28].

In this study, we performed radiolabeling and quality control studies with [^{99m}Tc]Tc for the usability of 6-MP as tumor diagnostic agent without long labeling process, heating, boiling, and purification.

Effect of reducing agent type and amount on radiolabeling of [^{99m}Tc]Tc-6-MP: In the +7 oxidation level, [^{99m}Tc]Tc was eluted from the

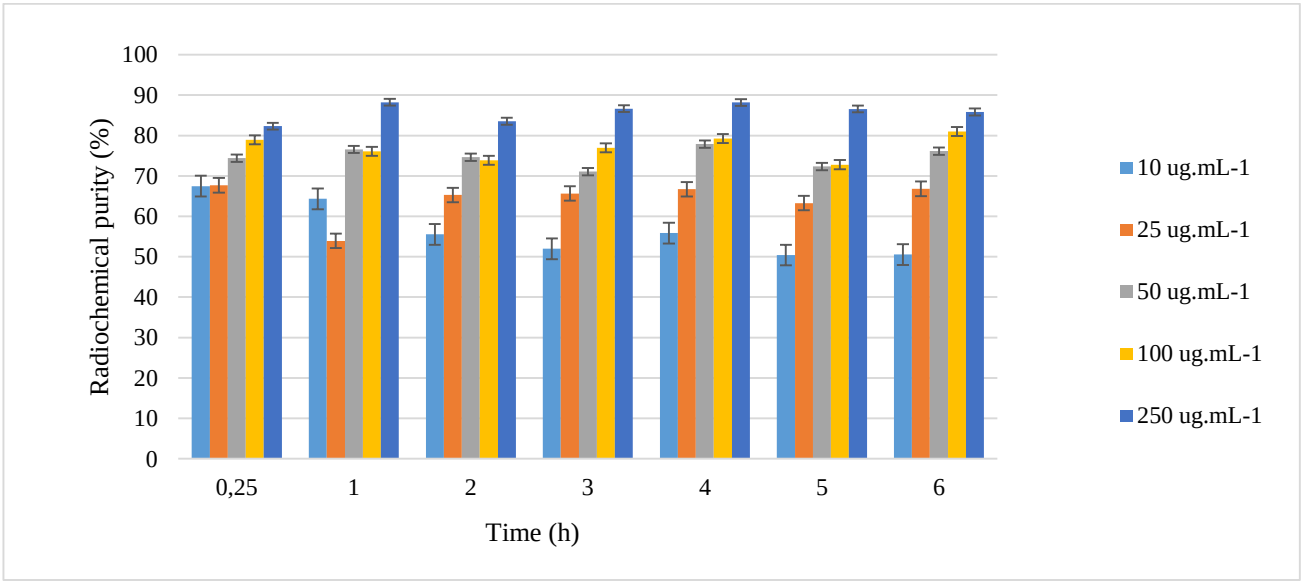

Figure 1. Radiochemical purity of [^{99m}Tc]Tc-6-MP with different amounts of stannous chloride.

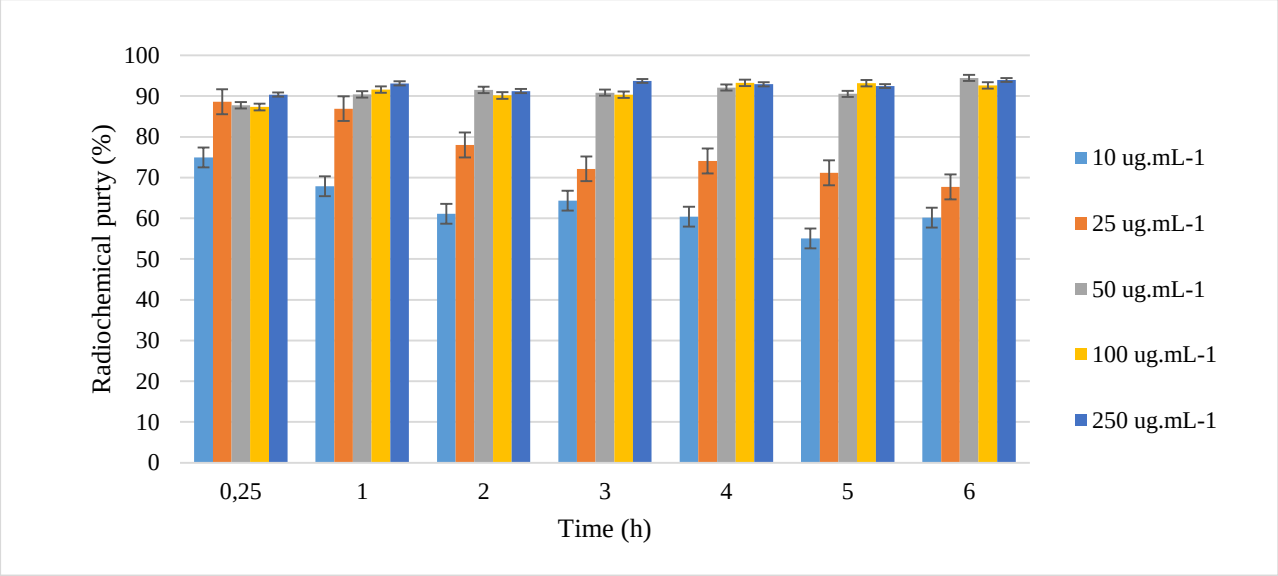

Figure 2. Radiochemical purity of [^{99m}Tc]Tc-6-MP with different amounts of stannous tartrate.

[^{99}Mo]Mo/[^{99m}Tc]Tc generator. When added directly, [^{99m}Tc]Tc cannot be labeled with any component. To form compounds with the ligand and produce the radiopharmaceutical, [^{99m}Tc]Tc must be lowered to +4/+5 oxidation levels prior to radiolabeling. The researchers have used a variety of reduction agents for this purpose, including stannous chloride, stannous tartrate, sulphonic acid, sodium dithionite, sodium borohydride, formamidine, hydrohalic acids, and others. Stannous salts are frequently used as a reductant within them due to its non-toxic and stable characteristics [9,29].

Labeling tests were carried out with the identical concentrations of active component (6-MP), reducing agent (stannous chloride or stannous tartrate), and radionuclide ([^{99m}Tc]Tc) in order to analyze the influence of reducing agent type and amount on RP. Two formulation groups were formed. The first group uses stannous chloride as a reducing agent, while the second uses stannous tartrate. Figure 1 and Figure 2 show comparative findings for both formulations.

As seen in Figure 1, in all added amounts of stannous chloride, the RP could not exceed 90%.

The RP of a radiopharmaceutical should be ≥ 90% to have high image quality and not retain radiochemical impurities in non-target tissues [30].

As seen in Figure 2, the RP of [^{99m}Tc]Tc-6-MP increased above 90% with the addition of increasing amounts of stannous tartrate to the radiolabeled complex. According to the results, it was selected formulations prepared with 50-250 µg stannous tartrate for further studies. Also, it is crucial to note that using more reducing agent than necessary when radiolabeling [^{99m}Tc]Tc to create [^{99m}Tc]Tc complexes is highly advised [31].

Effect of antioxidant agent on radiolabeling of [^{99m}Tc]Tc-6-MP: Auto radiolysis may occur during the produce and storage of [^{99m}Tc]Tc radiopharmaceuticals as a result of the water's natural degradation by ionizing radiation [32]. The RP of radiopharmaceuticals is reduced by this decomposition. As a result, using a stabilizer to decrease auto radiolysis is critical. Antioxidants including p-aminobenzoic acid, ascorbic acid, and gentisic acid are frequently used as radiolytic stabilizers [33]. In this study, we used ascorbic acid as antioxidant.

To determine the effect of antioxidant agent amount on RP, 0.025 mg and 0.050 mg ascorbic acid was added to the formulations prepared by adding 50-250 µg of stannous tartrate. The results were shown in Table 1–3.

Table 1. The radiochemical purity of [^{99m}Tc]Tc-6-MP including 50 µg stannous tartrate in the absence and presence of antioxidant agent.

Time (hour)	Radiochemical purity (%)		
	Absence of ascorbic acid	Ascorbic acid (mg)	
		0.025 mg	0.050 mg
0.25	87.76 ± 1.38	89.42 ± 2.56	89.61 ± 1.46
1	90.41 ± 2.39	90.35 ± 1.44	91.88 ± 2.33
2	91.53 ± 1.56	89.61 ± 2.09	91.17 ± 2.04
3	90.85 ± 1.07	92.51 ± 2.16	88.45 ± 1.56
4	92.10 ± 2.72	90.46 ± 1.38	90.36 ± 1.48
5	90.56 ± 1.67	91.25 ± 1.05	91.06 ± 3.64
6	94.45 ± 2.55	91.36 ± 2.34	90.37 ± 2.02

Table 2. The radiochemical purity of [^{99m}Tc]Tc-6-MP including 100 µg stannous tartrate in the absence and presence of antioxidant agent.

Time (hour)	Radiochemical purity (%)		
	Absence of ascorbic acid	Ascorbic acid (mg)	
		0.025 mg	0.050 mg
0.25	87.32 ± 1.46	89.27 ± 1.02	90.05 ± 1.24
1	91.59 ± 2.58	92.31 ± 1.88	91.69 ± 1.03
2	90.16 ± 1.33	89.20 ± 1.20	92.48 ± 2.55
3	90.36 ± 2.05	92.50 ± 2.43	91.80 ± 1.87
4	93.26 ± 3.45	93.53 ± 3.14	92.14 ± 2.40
5	93.19 ± 2.97	92.34 ± 2.47	93.65 ± 3.54
6	92.62 ± 2.15	92.06 ± 1.65	92.70 ± 1.30

Table 3. The radiochemical purity of [^{99m}Tc]Tc-6-MP including 250 µg stannous tartrate in the absence and presence of antioxidant agent.

Time (hour)	Radiochemical purity (%)		
	Absence of ascorbic acid	Ascorbic acid (mg)	
		0.025 mg	0.050 mg
0.25	90.37 ± 2.06	90.69 ± 0.86	93.45 ± 2.14
1	93.12 ± 2.74	91.43 ± 1.38	94.09 ± 2.98
2	91.23 ± 1.95	92.76 ± 1.47	94.90 ± 1.83
3	93.72 ± 1.87	92.94 ± 2.30	95.29 ± 2.07
4	92.91 ± 2.14	93.25 ± 2.76	97.48 ± 3.25
5	92.45 ± 1.65	92.37 ± 2.49	96.75 ± 2.68
6	93.94 ± 2.43	93.86 ± 3.63	96.03 ± 1.84

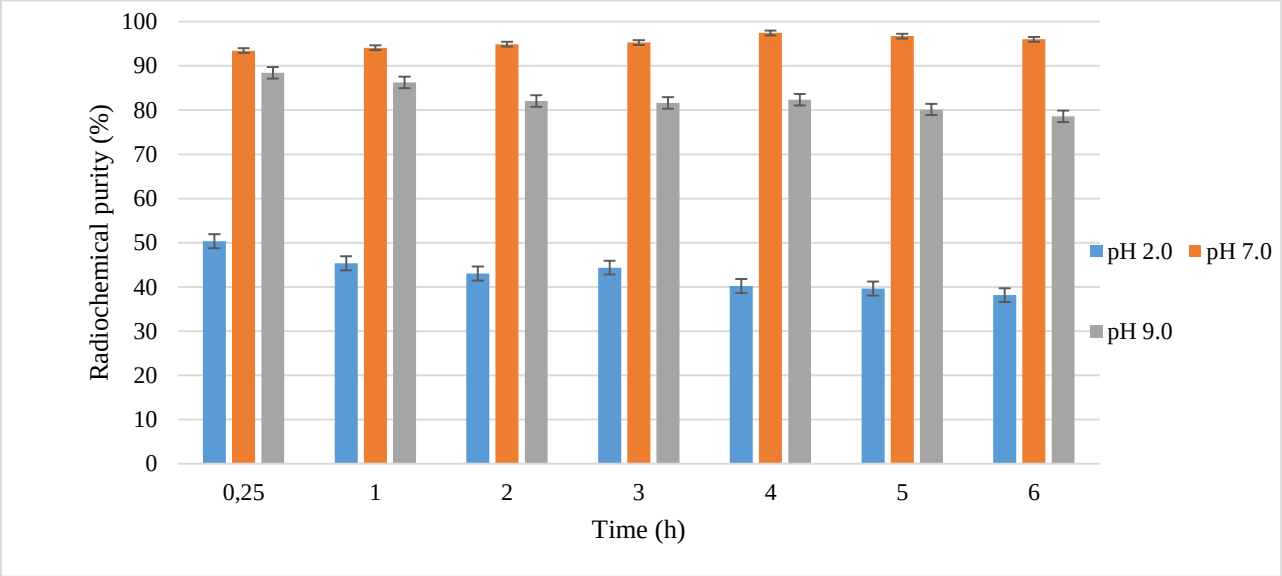

Figure 3. Effect of pH on radiochemical purity of [^{99m}Tc]Tc-6-MP.

According to Table 3, maximum labeling efficiency (>93%) was obtained with 250 µg stannous tartrate, 0.050 mg ascorbic acid included informulation and did not alter much after 6 h at room temperature (p>0.05).

Effect of pH on radiolabeling of [^{99m}Tc]Tc-6-MP: Although blood has a high buffering capacity and radiopharmaceuticals are small-volume preparations, the ideal pH for these substances is 7.4. However, pH values can potentially range from 2 to 9. This is because blood has such a great buffering capacity [18]. The impact of pH on labeling effectiveness was therefore examined for pH 2 to 9. In this experiment, the pH of the reaction medium played a significant role (Figure 3).

While keeping the other reaction parameters constant, substantial changes in labeling effectiveness were detected when the pH of the reaction was altered from 2 to 9. The pH of [^{99m}Tc]Tc-6-MP was shown to be optimum at 7.0 and [^{99m}Tc]Tc-6-MP was found stable up to 6-h (p>0.05).

Effect of incubation time on radiolabeling of [^{99m}Tc]Tc-6-MP: The incubation time of [^{99m}Tc]Tc-6-MP was determined how long after preparation the radiopharmaceutical was suitable for use in nuclear medicine. According to the obtained result which was shown in Table 4, the labeling efficiency of [^{99m}Tc]Tc-6-MP was above 90% in 5-min after labeling. The ideal radiolabeling efficiency (>93%) was observed after a 15-min incubation period, whereas incubation for longer periods did not result in significant changes (p<0.05).

Table 4. Effect of incubation time on radiochemical purity of [^{99m}Tc]Tc-6-MP.

Time (min)	Radiochemical purity (%)
5	90.52 ± 1.36
15	93.45 ± 2.14
30	93.53 ± 3.52
45	93.86 ± 2.10
60	94.09 ± 2.98

Effect of dose amount on radiolabeling of [^{99m}Tc]Tc-6-MP: The labeling studies of [^{99m}Tc]Tc-6-MP were carried out with 37 MBq of [^{99m}Tc]Tc in order to ensure the radiation safety of the staff and the surrounding region. Because radiopharmaceutical research on

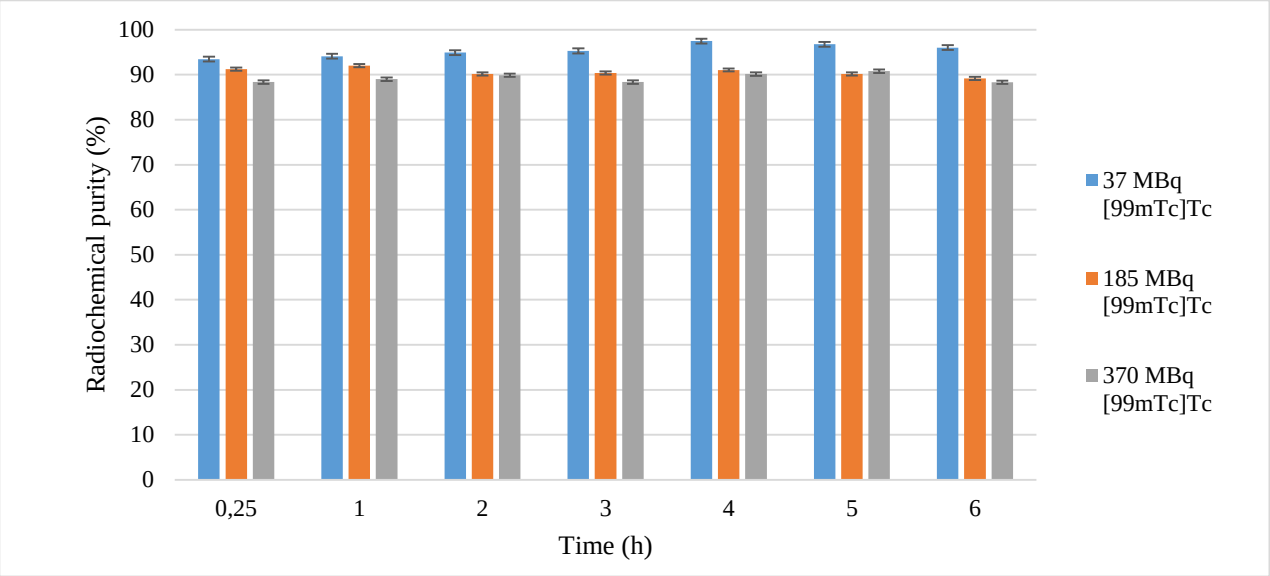

Figure 4. Effect of radiation dose on radiochemical purity of [^{99m}Tc]Tc-6-MP.

humans necessitates greater radiation doses, the RP of [^{99m}Tc]Tc-6-MP was also examined at 185 and 370 MBq radiation doses in addition to the 37 MBq radiation dosage and the results were given in Figure 4.

As a result of increasing the amount of radioactivity 10-folds, the RP value was found over 88%. As shown in Figure 4, with increased the radioactivity amount, there was a slight decrease in RP ($p>0.05$). Molar activity (A_m) and specific activity (A_s) are crucial properties for the development of novel radiopharmaceuticals [34]. The findings support [^{99m}Tc]Tc-6-MP's suitability as a radiopharmaceutical in nuclear medicine.

In vitro stability of [^{99m}Tc]Tc-6-MP: The *in vitro* stability of new radiopharmaceuticals is one of their major limitations. Thus, [^{99m}Tc]Tc-6-MP was incubated in SF in order to evaluate the complex stability. The results showed that [^{99m}Tc]Tc-6-MP was highly stable and RP was found >90% for 6-h in SF ($p>0.05$) (Figure 5).

Quality control of [^{99m}Tc]Tc-6-MP: High-performance liquid chromatography, RTLC, and/or gas chromatography can be utilized for the quality control of radiopharmaceutical [35]. In order to examine the RP of [^{99m}Tc]Tc-6-MP, RTLC approach was employed in this study because it is quickly and safely. To identify and measure the levels of radioactive impurities, selected mobile phases and stationary phases were given in Table 5.

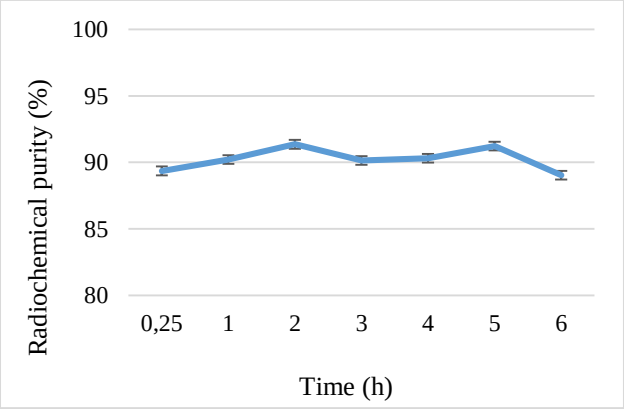

Figure 5. The stability of [^{99m}Tc]Tc-6-MP in saline up to 6 hours.

Using selected mobile and stationary phases, the RTLC chromatogram of [^{99m}Tc]Tc-6-MP was presented in Figure 6. Under optimized conditions, the RP of [^{99m}Tc]Tc-6-MP was over 90%.

Table 5. Rf values of [^{99m}Tc]Tc-6-MP in selected mobile and stationary phases.

	Whatman 3MM	**ITLC-SG**
	Acetone (100%)	**Acetonitrile/ Water/Trifluoroacetic acid (50/25/1.5)**
Free-[^{99m}Tc]Tc	0.9-1.0	0.9-1.0
R/H-[^{99m}Tc]Tc	0.0-0.1	0.0-0.1
[^{99m}Tc]Tc-6-MP	0.0-0.1	0.9-1.0

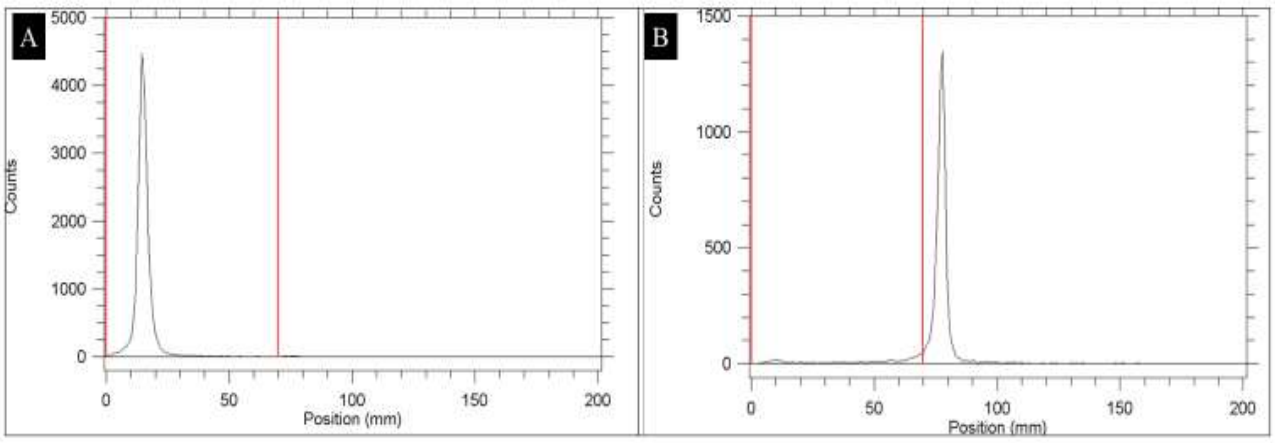

Figure 6. RTLC chromatogram of [^{99m}Tc]Tc-6-MP in different mobile phases: A: Acetone, B: CN/W/TFA (50/25/1.5).

Partition coefficient study of [^{99m}Tc]Tc-6-MP: The distribution of a molecule in the aqueous and organic phases at equilibrium is known as the n-octanol/PBS partition coefficient (log*P*), which gives important data for the *in vivo* behavior of the drug in drug development studies [20]. A gamma counter was used to detect the log*P* of the [^{99m}Tc]Tc-6-MP in this study. The log*P* of the [^{99m}Tc]Tc-6-MP were found to be -0.021 ± -0.001. According to result, the radiolabeled molecule has slightly polar properties (log*P*<1).

Conclusions

In this study, we showed that 6-MP can be radiolabeled with [^{99m}Tc]Tc with a high labeling efficiency (>90%) *via* RTLC technique. The produced complex was highly stable, with labeling efficiency continuing up to 6-h. With 250 µg stannous tartrate, 0.050 mg ascorbic acid, and 37 MBq [^{99m}Tc]$NaTcO_4$ containing formulations at pH 7.0 at room temperature, the highest RP was achieved. Also, further studies with [^{99m}Tc]Tc-6-MP are in progress in order to evaluate tumor cell binding capacity, biodistribution and imaging of the complex in experimental animals.

Acknowledgement: *The authors thank the Nuclear Medicine Department at Ege University, Türkiye, for providing the [^{99m}Tc]$NaTcO_4$.*

Funding: *TLC Scanner was funded by T.R. Prime Ministry State Planning Organization Foundation Grant Project Number: 09DPT001.*

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

Ethical statement: *Ethics committee decision was not taken as it was a laboratory study.*

References

[1] Siegel RL, Miller KD. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7–30.

[2] Ferlay J, Colombet M, Soerjomataram I, et al. Cancer statistics for the year 2020: An overview. Int J Cancer. 2021;10.1002/ijc.33588.

[3] Ekinci M, Santos-Oliveira R, İlem-Özdemir D. Biodistribution of 99mTc-PLA/PVA/Atezolizumab nanoparticles for non-small cell lung cancer diagnosis. Eur J Pharm Biopharm. 2022;176:21–31.

[4] Payolla FB, Massabni AC, Orvig C. Radiopharmaceuticals for diagnosis in nuclear medicine: A short review. Eclet Quim. 2019;44(3):11–9.

[5] Ge J, Zhang Q, Zeng J, et al. Radiolabeling nanomaterials for multimodality imaging: New insights into nuclear medicine and cancer diagnosis. Biomaterials. 2020;228:119553.

[6] Li H, Liu Z, Yuan L, et al. Radionuclide-based imaging of breast cancer: State of the art. Cancers (Basel). 2021;13(21):5459.

[7] Jafari SH, Saadatpour Z, Salmaninejad A, et al. Breast cancer diagnosis: Imaging techniques and biochemical markers. J Cell Physiol. 2018;233(7):5200-13.

[8] DeCesare A, De Vincentis G, Gervasi S, et al. Single-photon-emission computed tomography (SPECT) with technetium-99m sestamibi in the diagnosis of small breast cancer and axillary lymph node involvement. World J Surg. 2011;35(12):2668-72.

[9] 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.

[10] Papagiannopoulou D. Technetium-99m radiochemistry for pharmaceutical applications. J Label Compd Radiopharm. 2017;60(11):502–20.

[11] ME M, AS A, Y M. Cytotoxicity of 6-mercaptopurine via loading on pva-coated magnetite nanoparticles delivery system: a new era of leukemia therapy. J Nanomed Nanotechnol. 2018;9:6.

[12] Selvaraj V, Alagar M, Hamerton I. Analytical detection and biological assay of antileukemic drug using gold nanoparticles. Electrochim Acta. 2006;337(1–2):275–81.

[13] Bariyanga J, Luyt AS. Synthesis, fourier transform infrared, nuclear magnetic resonance and thermal analysis of sodium and platinum complexes of 6-mercaptopurine. J Mol Struct. 2001;559(1–3):49–54.

[14] Dorniani D, Hussein MZ Bin, Kura AU, et al. Preparation and characterization of 6-mercaptopurine-coated magnetite nanoparticles as a drug delivery system. Drug Des Devel Ther. 2013;7:1015–26.

[15] Nezhad-Mokhtari P, Ghorbani M, Mahmoodzadeh F. Smart co-delivery of 6-mercaptopurine and methotrexate using disulphide-based PEGylated-nanogels for effective treatment of breast cancer. New J Chem. 2019;43:12159–67.

[16] Maioli C, Lucignani 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.
[17] 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.
[18] İlem-Özdemir D, Ekinci M, Özgenç E, et al. 99mTc-exendin-4: Radiolabeling and quality control studies of glucagon-like peptide analog. Exp Biomed Res. 2022;5(3):235–43.
[19] Ilem-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.
[20] Ekinci M, Öztürk AA, Santos-Oliveira R, et al. The use of Lamivudine-loaded PLGA nanoparticles in the diagnosis of lung cancer: Preparation, characterization, radiolabeling with 99mTc and cell binding. J Drug Deliv Sci Technol. 2022;69:103139.
[21] Croce CM. Oncogenes and cancer. N Engl J Med. 2008;358:502-11.
[22] Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71:209-49.
[23] Pijeira MSO, Viltres H, Kozempel J, et al. Radiolabeled nanomaterials for biomedical applications: radiopharmacy in the era of nanotechnology. EJNMMI Radiopharmacy and Chemistry. 2022;7:8.
[24] Diniz SOF, Siqueira CF, Nelson DL, et al. Technetium-99m ceftizoxime kit preparation. Brazilian Arch. Biol. Technol. 2005;48:89–96.
[25] Banerjee I, Behera A, De K, et al. Synthesis, characterization, biodistribution and scintigraphy of 99mTc-paclitaxel: a potential tracer of paclitaxel. J Radioanal Nucl Chem.2015;304:633–43.
[26] Adamson PC, Balis FM, Hawkins ME, et al. Desulfuration of 6-mercaptopurine. The basis for the paradoxical cytotoxicity of thiopurines in cultured human leukemic cells. Biochem Pharmacol. 1993;46(9):1627–36.
[27] Guarino A, Shukla SK, Castelli L, et al. Uptake of 67Ga-6-mercaptopurines in Morris hepatoma-3924 A. Eur J Nucl Med. 1979;4:211–5.
[28] Hunt FC, Booker J, McLaughlin AF, et al. Evaluation of a new bile agent technetium-99m-mercaptopurine. In: Newton PJF, editor. 5 Annual Scientific Meeting, Sydney, Australia: Australian and New Zealand Society of Nuclear Medicine, North Melbourne (Australia); 1974. p. 7–5. Available from: https://inis.iaea.org/collection/NCLCollectionStore/_Public/05/141/5141350.pdf?r=1
[29] Shahzad K, Majid ASA, Khan M, et al. Recent advances in the synthesis of (99mTechnetium) based radio-pharmaceuticals. Rev Inorg Chem. 2021;41(3).
[30] Elitez Y, Ekinci M, Ilem-Ozdemir D, et al. Tc-99m radiolabeled alendronate sodium microemulsion: characterization and permeability studies across caco-2 cells. Curr Drug Deliv. 2018;15(3):342–50.
[31] Costa B, Ilem-Özdemir D, Santos-Oliveira R. Technetium-99m metastable radiochemistry for pharmaceutical applications: old chemistry for new products. J Coord Chem. 2019;72(11):1759–84.
[32] Tubergen K, Corlija M, Volkert WA, et al. Sensitivity of technetium-99m-d,l-HMPAO to radiolysis in aqueous solutions. J Nucl Med. 1991;32(1):111–5.
[33] Ekinci M, Ilem-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] Luurtsema G, Pichler V, Bongarzone S, et al. EANM guideline for harmonisation on molar activity or specific activity of radiopharmaceuticals: impact on safety and imaging quality. EJNMMI Radiopharm Chem. 2021;6:34.

[35] Chen F, Decristoforo C, Rohrbacher B, et al. A simple two-strip method to determine the radiochemical purity of technetium-99m mercaptoacetyltriglycine. Eur J Nucl Med. 1993;20(4):334–8.