**Exp Biomed Res 2025; 8(1): 1-7** **https://doi.org/10.30714/j-ebr.2025.231**

**Experimental Biomedical Research** *Original article*

# Radiolabeling and quality control studies of [$^{99m}$Tc]Tc-dexketoprofen trometamol for inflammation imaging

**Meliha Ekinci[1*], Ahmet Alper Öztürk[2], Derya İlem Özdemir[1]**

[1]*Department of Radiopharmacy, Faculty of Pharmacy, Ege University, Izmir, Türkiye*

[2]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Anadolu University, Eskisehir, Türkiye*

## ABSTRACT

**Aim:** To radiolabel dexketoprofen trometamol (DT) with [$^{99m}$Tc]Tc for developing inflammation imaging agent.

**Method:** For this purpose, DT was radiolabeled using [$^{99m}$Tc]Tc, and radioactive thin layer chromatography (RTLC) was used in the quality control studies of [$^{99m}$Tc]Tc-DT. In addition, the effects of quality control factors (reducing agent, incubation time, pH value) on radiolabeling were studied. Finally, the stability study of [$^{99m}$Tc]Tc-DT was performed and the partition coefficient value of [$^{99m}$Tc]Tc-DT was calculated.

**Results:** Based on the results, a new, simple, and fast direct technique was used for producing [$^{99m}$Tc]Tc-DT with over 92% labeling efficiency after 15 minutes of pH 9.0 incubation. 37 MBq [$^{99m}$Tc]Tc, 10 µg of stannous chloride dihydrate (reducing agent), and 250 µg of DT were utilized to produce the ideal radiolabeling conditions. According to the RTLC experiments, [$^{99m}$Tc]Tc-DT remained stable at room temperature for six hours. The [$^{99m}$Tc]Tc-DT log*P* has been determined to be -0.48 ± 0.02.

**Conclusions:** Based on the acquired results, [$^{99m}$Tc]Tc-DT has the potential to be an imaging agent for inflammation. Further studies are in progress, such as biodistribution of [$^{99m}$Tc]Tc-DT.

**Keywords:** Dexketoprofen trometamol, radiolabeling, radiopharmaceuticals, technetium-99m.

✉ *Meliha Ekinci**

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

*E-mail: melihaekinci90@gmail.com*

*Received: 2024-07-03 / Revisions: 2024-09-11*

*Accepted: 2024-10-06 / Published: 2025-01-01*

## 1. Introduction

Infection is frequently brought on by the invasion and growth of microorganisms including viruses, parasites, and bacteria that are not typically found in the body. Inflammation is used to describe the infected tissues' inflammation, which is a main sign of the invasion and proliferation of microorganisms [1]. Imaging an inflammatory location enables diagnosis and therapy. It is well established that the ability to detect the cyclooxygenase (COX) enzyme can be a helpful tool for tracking inflamed tissues and detecting chronic inflammation [2].

'Anti-inflammatory analgesics' refers to non-steroidal anti-inflammatory medicines (NSAIDs), the most widely used medications for treating pain [3]. NSAIDs have an anti-inflammatory activity by directly suppressing prostaglandin synthesis by inhibition of the COX enzyme, which therefore results in a suppression of inflammation [4]. Dexketoprofen trometamol (DT) belongs to the NSAID group and is used for the symptomatic treatment of pain. DT, which is an active optical isomer of ketoprofen, is

produced as the water-soluble salt of dexketoprofen [5].

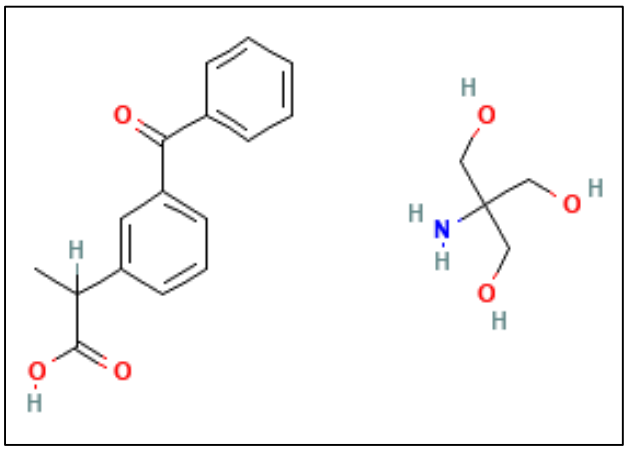

**Figure 1** shows the chemical structure of dexketoprofen trometamol (DT).

There are many diagnostic imaging techniques, including computed tomography, magnetic resonance imaging, ultrasonography, single-photon emission computed tomography (SPECT), and positron emission tomography (PET). Nuclear imaging techniques (PET, SPECT) are of particular interest due to the improved monitoring of the infectious process and higher specificity of imaging results. To precisely identify sites of infection and inflammation, these techniques utilize radiolabeled NSAIDs or antibiotics [6-8]. There have been numerous radiodiagnostic agents for infection and inflammation with PET and SPECT described up to now [4,9-12]. One of the earliest and most widely used radiopharmaceuticals for the detection of inflammation is [$^{67}$Ga]Ga-citrate [4]. Furthermore, a large number of radiolabeled COX-2 selective inhibitors have been documented in the literature [13,14]. [$^{99m}$Tc]Tc-diflunisal was synthesized by Van Sorge and his collaborator [13], and Yang *et al.* [14] synthesized [$^{99m}$Tc]Tc-labeled Celebrex which were non-selective COX-inhibitor. Despite the radiolabeling and research of a number of COX-2 inhibitors for COX-2 molecular imaging, we still need to find an appropriate radiopharmaceutical for imaging of inflammation.

Of all the radioisotopes used in nuclear medicine for diagnostics, [$^{99m}$Tc]Tc is the most suitable. The patient is subjected to a very low radiation dosage since [$^{99m}$Tc]Tc has a physical half-life of six hours, only releases gamma radiation (140 KeV) [15,16].

Radiopharmaceuticals are products of combining pharmaceutical and radioactive components [17]. In this study, to form a radiopharmaceutical, DT was selected as pharmaceutical part and [$^{99m}$Tc]Tc was selected as radioactive part. The purpose of this work is to produce a new radiopharmaceutical for inflammatory imaging. With the proper conditions, DT was labeled with [$^{99m}$Tc]Tc for this purpose. Within the framework of the study, radioactive thin layer chromatography (RTLC) was used to assess the labeling efficiency and stability of [$^{99m}$Tc]Tc-DT.

## 2. Materials and methods

***2.1. Material:*** DT was a present from Abdi Ibrahim (Turkey). Stannous chloride dihydrate was acquired from Sigma-Aldrich (USA). [$^{99m}$Tc]$NaTcO_4$ was eluted from the [$^{99}$Mo]Mo/[$^{99m}$Tc]Tc generator (Ege University, Turkey). All chemicals were acquired from Merck (Germany).

***2.2. Radiolabeling study:*** To optimize of reaction parameters for the radiolabeling of [$^{99m}$Tc]Tc-DT, a controlled series of experiments was carried out. For this purpose, 250 µg of DT in saline solution (SF) in four individual vials. Then, 5 to 20 µg of stannous chloride dihydrate were added in each vial. The radiolabeling was carried out at pH value of 5.0–11.0. After addition of components, 37 MBq of [$^{99m}$Tc]$NaTcO_4$ in SF solution was added, and

vials were vortexed for 1 min. Then, the radiolabeled system was incubated for 10–60 min. Every vial used in these studies had a total volume of 1.0 ± 0.1 mL.

***2.3. SF stability study:*** For the stability of [$^{99m}$Tc]Tc-DT in SF, 0.1 mL of [$^{99m}$Tc]Tc-DT was added to 0.4 mL of SF solution. The radiolabeled complex was incubated, and radiochemical purity (RCP, %) of [$^{99m}$Tc]Tc-DT were performed up to six hours.

**2*.4. Quality control study*:** The RCP (%) of radiolabeled complex was determined with the help of RTLC. A drop of [$^{99m}$Tc]Tc-DT was spotted on the chromatographic papers having dimensions of 1.5 × 8 cm each. To measure free-[$^{99m}$Tc]Tc, Whatman No. 3 plates were employed as the stationary phase and 100% acetone as the mobile phase. Reduced/Hydrolized (R/H)-[$^{99m}$Tc]Tc was assessed using ITLC-SG sheets prepared in 0.5 N NaOH. Following chromatographic separation, the radioactivity on the papers was evaluated using a TLC scanner, and the RCP (%) of [$^{99m}$Tc]Tc-DT was calculated using Equation 1:

RCP (%) = 100 – (Free-[$^{99m}$Tc]Tc (%) + Colloidal-[$^{99m}$Tc]Tc (%)) (Eq.1)

***2.5. Lipophilicity study:*** For the partition coefficient evaluation of [$^{99m}$Tc]Tc-DT, n-octanol (500 µL), phosphate buffer solution (PBS, pH: 7.4; 500 µL), and [$^{99m}$Tc]Tc-DT (50 µL) were included to an eppendorf centrifuge, mixed for one minute, and centrifuged for thirty minutes at 5000 rpm. The mixture divided into two phases after centrifugation, and the activity of phases was counted using a gamma counter. The log*P* value of [$^{99m}$Tc]Tc-DT was calculated using Equation 2:

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

***2.6. Statistical analysis:***

All the data were calculated using Microsoft Excel. The *t* test was used to determine the statistical significance. There were differences that were considered significant at the 95% confidence level ($p$>0.05).

## 3. Results and Discussion

***3.1. Radiolabeling study:*** In order to assess the suitability of DT as an imaging agent for inflammation, we carried radiolabeling and quality control studies using [$^{99m}$Tc]Tc. To obtained maximum radiochemical purity (RCP) of [$^{99m}$Tc]Tc-DT, a controlled series of radiolabeling procedures were performed, which, as Figure 2 illustrates, provided different outcomes. The optimum set of reaction conditions yielded an RCP of >92%, although throughout the process of optimizing the reactions, every parameter was found to have a significant effect on radiolabeling yield.

***3.2. Reducing agent amount study:*** [$^{99m}$Tc]Tc is eluted from the [$^{99}$Mo]Mo/[$^{99m}$Tc]Tc generator in the +7-oxidation state, which cannot be labeled with any ligand. Lowering the oxidation levels of [$^{99m}$Tc]Tc is necessary for it to form a compound with the ligand and produce the radiopharmaceutical. Because stannous chloride dihydrate is stable and non-toxic, it is widely employed as a reductant within different reducing agents such as sodium borohydride, sodium dithionite, stannous chloride, and stannous tartrate [18].

Figure 2A showed the influence of reducing agent amount on the RCP of [$^{99m}$Tc]Tc-DT. Stannous chloride dihydrate in neutral solution (5–20 µg) was used as a reducing agent. The maximum RCP (>92%) was obtained with 10 µg stannous chloride dihydrate, however, the reaction produced a decreased RCP at both lower and higher concentrations. A radiopharmaceutical's RCP must be greater than 90% in order to produce high-quality images without retaining radiochemical impurities in non-target tissues [19].

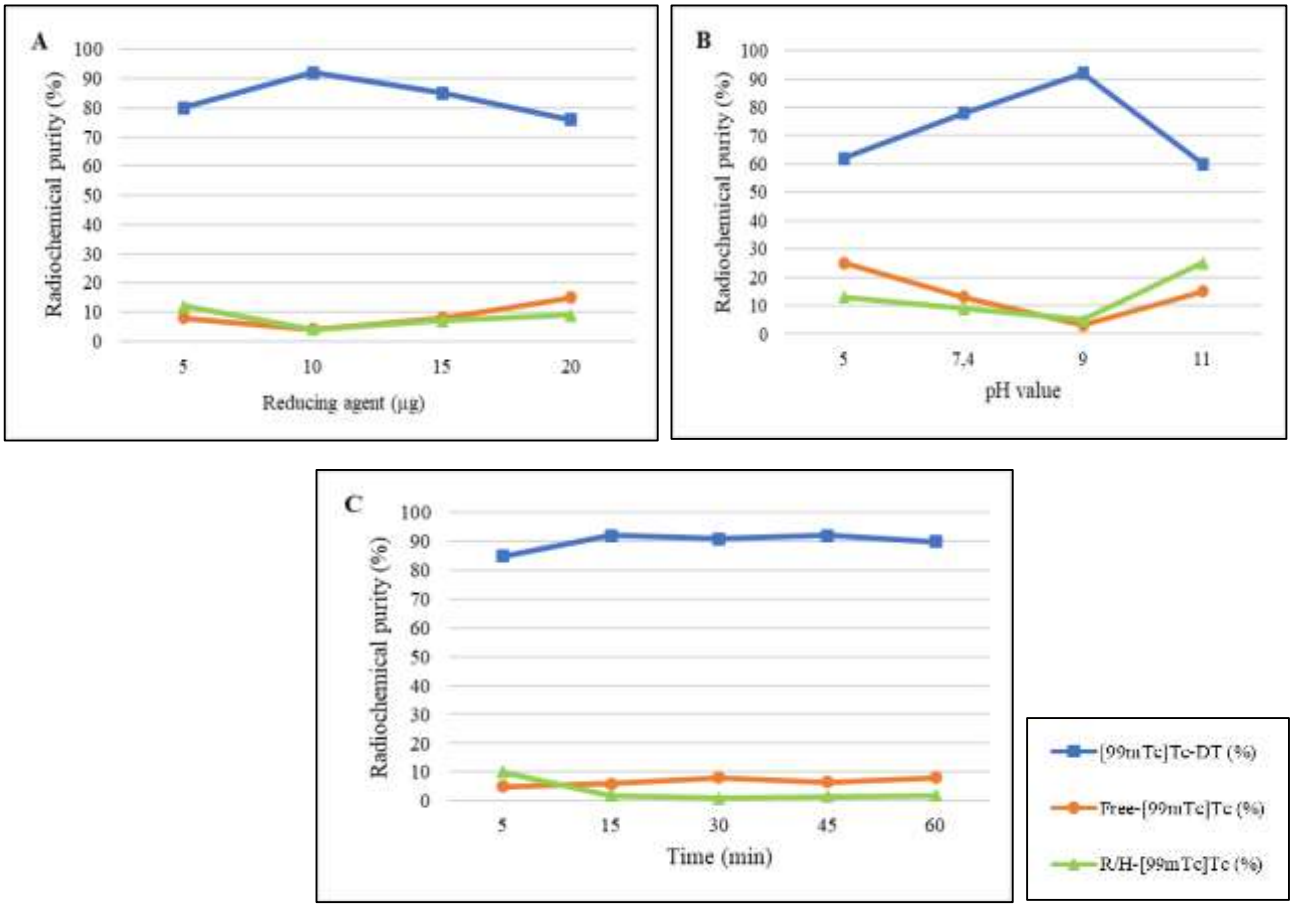


**Figure 2.** The effect of A) reducing agent amount, B) pH value and C) incubation time.

***3.3. pH value study:*** Radiopharmaceuticals should be prepared in an aqueous solution with a pH range compatible with blood in order to be injected into people or animal models. pH levels may vary even though 7.4 is the optimal pH for radiopharmaceuticals. This is due to the small volume characteristics of radiopharmaceuticals and the high buffering capacity of blood [20]. For this purpose, the effect of pH on radiolabeling was therefore studied for pH 5.0 to 11.0. In this experiment, the pH value played a significant role (Figure 2B). [$^{99m}$Tc]Tc-DT was shown to be stable for up to 6 hours, with an optimal pH of 9.0 ($p$>0.05).

***3.4. Incubation time study:*** The duration of incubation of [$^{99m}$Tc]Tc-DT is used to determine when the radiopharmaceutical was appropriate for use following manufacture. Based on the chromatographic study, [$^{99m}$Tc]Tc-DT was formed at 15 min reaction period. As seen in Figure 2C, there was no change in the radiochemical yield above this point ($p$<0.05).

***3.5. In vitro* stability study:** A significant limitation on novel radiopharmaceuticals is their stability. Thus, the stability of [$^{99m}$Tc]Tc-DT in SF was studied. The stability studies showed that RCP was >90% for six hours in SF ($p$>0.05) and that [$^{99m}$Tc]Tc-DT was very stable (Figure 3).

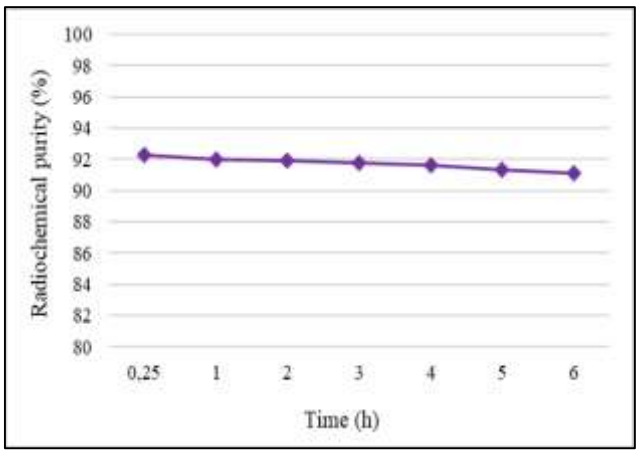


**Figure 3.** The stability of [$^{99m}$Tc]Tc-DT in SF.

***3.6. Quality control study:*** In this work, the RTLC was used to investigate the RCP of [$^{99m}$Tc]Tc-DT due to its speed and safety. Table 1 presented specific phases that were both mobile and stationary.

**Table 1.** R*f* values of [$^{99m}$Tc]Tc-DT.

| **Variables** | **Whatman No. 3** | **ITLC-SG** |
|---|---|---|
| | **Acetone** | **0.5 N NaOH** |
| **Free-[$^{99m}$Tc]Tc** | 0.8-1.0 | 0.8-1.0 |
| **Colloidal [$^{99m}$Tc]Tc** | 0.0-0.2 | 0.0-0.2 |
| **[$^{99m}$Tc]Tc-DT** | 0.0-0.2 | 0.8-1.0 |

The RCP (%) of [$^{99m}$Tc]Tc-DT, free-[$^{99m}$Tc]Tc and colloidal [$^{99m}$Tc]Tc was determined using ITLC-SG papers and Whatman No. 3. As seen in Figure 4A, the free-[$^{99m}$Tc]Tc was migrated along with solvent front (Rf = 0.8-1.0; 4.12 ± 0.28%) leaving bound and R/H-[$^{99m}$Tc]Tc at application point (Rf = 0.0–0.2; 96.20 ± 1.25%). As seen in Figure 4B, the R/H-[$^{99m}$Tc]Tc remained at application point (Rf = 0.0-0.2; 4.25 ± 0.36%) and [$^{99m}$Tc]Tc-DT and free-[$^{99m}$Tc]Tc was migrated along with solvent front (Rf = 0.8–1.0; 96.05 ± 1.62%). Under optimized conditions, Figure 4 showed the [$^{99m}$Tc]Tc-DT chromatogram, which the RCP of [$^{99m}$Tc]Tc-DT was over 90%.

***3.7. Lipophilicity study:*** Radiopharmaceuticals' charge, size, mass, shape, and lipophilicity all have an impact on how soluble they are in aqueous solution. Lipophilicity, represented by log*P*, is a fundamental physicochemical property of all compounds, signifying the moiety's or molecule's affinity for a lipophilic environment. Additionally, lipophilicity is crucial for drug molecule absorption, distribution, and excretion. High water solubility, quick kidney excretion, and frequently inability to pass through the blood-brain barrier (BBB) are characteristics of polar substances [21]. In this study, the gamma counter provided a log*P* of -0.48 ± -0.02 for the [$^{99m}$Tc]Tc-DT. The radiolabeled compound displays somewhat polar characteristics, according to the result (log*P*<1).

In a study Khan *et al.* developed [$^{99m}$Tc]Tc-ibuprofen for the visualization of inflammation. According to obtained results, the best RCP (>94%) was achieved with 600 µg of ibuprofen, 300 MBq [$^{99m}$Tc]Tc, and 4 µg of stannous chloride at pH value 11.0 for 15 min. Biodistribution study showed promising inflamed to normal tissues ratio as 4.57 ± 0.56. The researchers concluded that [$^{99m}$Tc]Tc-ibuprofen could be promising agent for imaging of inflammation [1].

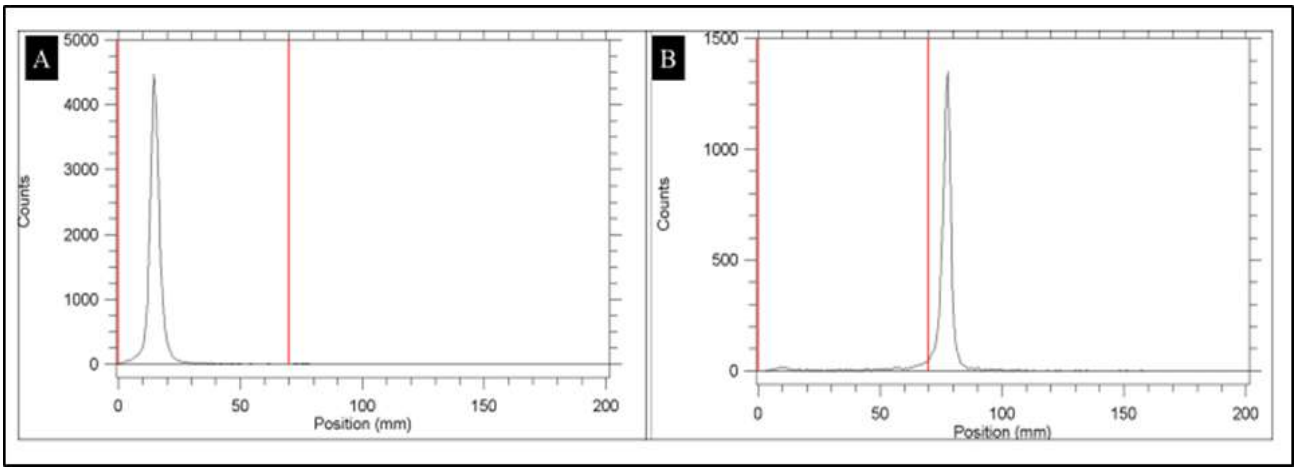


**Figure 4.** The chromatogram of [$^{99m}$Tc]Tc-DT in varying mobile and stationary phases: **A:** Acetone and Whatman No.3, **B:** 0.5 N NaOH and ITLC-SG.

***3.8. Conclusion:*** In summary, our study demonstrated that the RTLC approach can radiolabel DT with [$^{99m}$Tc]Tc with a high labeling effectiveness (>92%). The complex that developed was quite stable, and the radiolabeling efficiency maintained for six hours. The highest RCP was obtained with formulations comprising 10 µg stannous chloride dihydrate and 37 MBq [$^{99m}$Tc]$NaTcO_4$ at pH 9.0. Also, log*P* value of [$^{99m}$Tc]Tc-DT was calculated and found -0.48 ± -0.02, which is hydrophilic value. Additionally, the study has limitation. More research using [$^{99m}$Tc]Tc-DT is should conducted to assess the complex's biodistribution and imaging in test animals.

***Conflict of interest statement:*** *The authors declared no conflict of interest.*

***Acknowledgements:*** *The authors are grateful to the Nuclear Medicine Department at Ege University for [$^{99m}$Tc]Tc. This manuscript was edited by Paperpal for English grammar.*

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

***Ethical statement:*** *Ethics committee decision was not taken as it 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) 2025:*** *Author (s).*

## References

[1]Khan NU, 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(3): 3093–3100.

[2]Kyprianidou P, Tsoukalas C, Chiotellis A, et al. First example of well-characterized Re and $^{99m}$Tc tricarbonyl complexes of ciprofloxacin and norfloxacin in the development of infection-specific imaging agents. Inorganica Chim Acta. 2011; 370(1): 236-242.

[3]Zhang Z, Chen F, Shang L. Advances in antitumor effects of NSAIDs. Cancer Manag Res. 2018; 10: 4631–4640.

[4]Kniess T, Laube M, Wüst F, et al. Technetium-99m based small molecule radiopharmaceuticals and radiotracers targeting inflammation and infection. Dalton Trans (Cambridge, England: 2003). 2017; 46(42): 14435–14451.

[5]Öztürk AA, Yenilmez E, Arslan R, et al. Dexketoprofen trometamol loaded solid lipid nanoparticles (SLNs): Formulation, in vitro and in vivo evaluation. J Res Pharm. 2020; 24(1): 82-99.

[6]Fazlil A, Salouti M, Ahmadi G, et al. Radiolabeling of ceftriaxone with $^{99m}$Tc as a targeting radiopharmaceutical for Staphylococcus aureus detection in mouse model. Iran J Med Phys. 2012; 9(2): 103-110.

[7]Rasheed R, Naqvi SAR, Gillani SJH, et al. $^{99m}$Tc-tazobactam, a novel infection imaging agent: Radiosynthesis, quality control, biodistribution, and infection imaging studies. J Label Compd Radiopharm. 2017; 60(5): 242–249.

[8]Motaleb MA, Ibrahem IT, Ayoub VR, et al. Preparation and biological evaluation of ($^{99m}$)Tc-ropinirole as a novel radiopharmaceutical for brain imaging. J

Label Compd Radiopharm. 2016; 59(4): 147–152.

[9] Lambrecht FY, Durkan K, Yildirim Y, et al. Labeling of acetaminophen with I-131 and biodistribution in rats. Chem Pharm Bull. 2006; 54(2): 245–247.

[10] Ady J, Fong Y. Imaging for infection: from visualization of inflammation to visualization of microbes. Surg Infect 2014; 15(6): 700–707.

[11] Ilem-Özdemir D, Asikoglu M, Ozkilic H, et al. ($^{99m}$)Tc-Doxycycline hyclate: a new radiolabeled antibiotic for bacterial infection imaging. J Label Compd Radiopharm. 2014; 57(1): 36–41.

[12] Ilem-Ozdemir D, Asikoglu M, Ozkilic H, et al. Gamma scintigraphy and biodistribution of ($^{99m}$)Tc-cefotaxime sodium in preclinical models of bacterial infection and sterile inflammation. J Label Compd Radiopharm. 2016; 59(3): 109–116.

[13] van Sorge AA, van Etten RJ, Rehmann CJ, et al. $^{99m}$Tc-diflunisal and the human iris: topical application reveals localization. J Ocul Pharmacol Ther. 2002; 18(2): 185–195.

[14] Yang DJ, Bryant J, Chang JY, et al. Assessment of cyclooxygense-2 expression with $^{99m}$Tc-labeled celebrex. Anti-Cancer Drugs. 2004; 15(3): 255–263.

[15] Duatti A. Review on $^{99m}$Tc radiopharmaceuticals with emphasis on new advancements. Nucl Med Biol. 2021; 92: 202–216.

[16] Boschi A, Uccelli L, Martini P. A picture of modern Tc-99m radiopharmaceuticals: Production, chemistry, and applications in molecular imaging. App Sci. 2019; 9(12): 2526.

[17] Ekinci M, İlem-Özdemir D. Radyofarmasötikler ve teranostikler [Radiopharmaceuticals and theranostics]. J Lit Pharm Sci. 2021; 10(1): 119–132.

[18] El-kawy OA, Abdel-Razek AS, Sayed MS. Radiolabeling, biological evaluation and molecular docking of delafloxacin: a novel methicillin-resistant Staphylococcus aureus infection radiotracer. J Radioanal Nucl Chem. 2016; 308: 1081–1088.

[19] Ekinci M, Santos-Oliveira R, Ilem-Ozdemir D. Biodistribution of $^{99m}$Tc-PLA/PVA/Atezolizumab nanoparticles for non-small cell lung cancer diagnosis. Eur J Pharm Biopharm. 2022; 176: 21–31.

[20] 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): 874–884.

[21] 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 $^{99m}$Tc and cell binding. J Drug Deliv Sci Technol. 2022; 69: 103139.