Synthesis, Characterization, Biodistribution and Scintigraphic Evaluation of Technetium-99m (99mTc) Labeled Pazufloxacin (PZN): A Novel Infection Radiotracer

Syed Qaiser Shah* and Shahia Khattak

Biochemistry Section, Institute of Chemical Sciences, University of Peshawar, Peshawar, KPK, Pakistan

*Corresponding author: Prof. Dr. Syed Qaiser Shah, M.Sc, M.Phil, PhD, Professor & Head Biochemistry Section, University of Peshawar, Peshawar, KPK, Pakistan, Tel: 00 92 91 9216701-20, 0333 9254009; Fax: 00 92 91 9216447; E-mail: ssqaiser2002@yahoo.com

Received date: Jul 02, 2015, Accepted date: Aug 21, 2015, Publication date: Aug 25, 2015

Copyright: © 2015 Shah SQ, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License; which permits unrestricted use; distribution; and reproduction in any medium; provided the original author and source are credited.

Abstract

In this study tagging of 99mTc with PZN was examined with regards to different concentration of PZN, stannous chloride, 99mTc and pH ranges from 5.00-6.00. The suitability of the 99mTc labeled PZN was assessed in terms of percent radiochemical purity (%RCP) yield in normal saline at concentration value of the stannous chloride as 120 mg, stability in serum (in vitro) at 37°C at different intervals, in vitro up take by live and heat killed E. coli, biodistribution behavior in artificially E. coli (live and heat killed) infected animal model rat and scintigraphic accuracy in artificially E. coli infected animal model rabbit. Maximum %RCP yields of 98.25 ± 0.26% was observed after 30 min of labeling using 2 mg of PZN, 125 μg stannous chloride, 2.5 m Ci sodium pertechnetate and pH 5.3.

The labeled PZN showed stable profile in serum at 37°C with a total decay of 16.40 ± 0.18%. The labeled PZN showed 72.55 ± 0.90% maximum bacterial uptake after 90 min in live and in case of heat killed E. coli, no significant uptake was seen. The labeled PZN showed 14.75 ± 0.40% absorption and accumulation in the infected muscle of the rat infected with live E. coli and infected to normal muscle ratio was 6.1. Scintigraphically, it was observed that after few minutes, the activity accumulated in the infected muscle was more than the others sites. The instant investigation substantiated that labeling PZN through this technique as compared to 99mTc(V-N) and 99mTc(CO)3– showed almost analogous %RCP yield, stability in serum at 37°C, in vitro binding with E. coli, biodistribution profile in animal modal rats and scintigraphic accuracy in animal model rabbit.

Keywords: 99mTc-PZN; Escherichia coli (E. coli); Infection radiotracer

Introduction

In time diagnosis of infection and its discrimination from non-infectious site is challenging for clinicians but imperative for decisive planning and suitable treatment. Routine pathological tests can identify the pathogen which caused infection but cannot offer any trace to distinguish between infection and inflammation [1,2].

Detection and monitoring of a variety of infectious diseases can be accomplished using a range of diagnostic modalities like ultra-Sound (US), Computed Tomography (CT), Magnetic Resonance Imaging (MRI), etc. [3]. These investigative modalities have proven helpful in recognizing infections because of their exceptional resolution, which facilitate the clinicians to evaluate more precisely the internal sites of indistinct infection [4].

Nevertheless, these investigative modalities depend only on morphological alterations and consequently most anomalies can only be detect at complex stages of ailments and diagnosis of in time infections and its discrimination from inflammation or other involvements can be tricky [5].

However, functional imaging can in many situations offer helpful and corresponding diagnostic details. Nuclear medicine (NM) procedures in contrast to US, CT and MRI, can find out the site and degree of infection on the basis of physiologic and metabolic alterations [6].

Gallium-67(Ga-citrate) [7], Technetium-99 (99mTc) or Indium-111 (111In) labeled White Blood Cell (WBC) and peptides [8,9], have revealed some encouraging results with some disadvantages like accessibility, cumbersome labeling procedures and radiation burden.

Recently, 99mTc labeled antibiotics [10-15] have been explored for infection localization because of their high radiochemical yield, stability, binding with specific pathogen and localized imaging.

Nowadays, a need for a precise and specific infection imaging agent is still felt for in time identification of infection and its discrimination from non-infectious sites. In these lines, direct radiolabeling of a new quinolone antibiotic, Pazufloxacin (PZN) as shown in Figure 1. with 99mTc has been explored.

The rationale for PZN radiolabeling is its superior activity not only against gram-positive and gram-negative pathogen but also with other defiant strains. The higher activity of the PZN is due to the 1-aminocyclopropyl at C-10.

PZN inhibit the growth of bacteria via inhibition of bacterial DNA gyrase. These idiosyncratic characteristics posed the PZN, a drug of choice against E. coli when other quinolones have proven ineffective [16,17].
In the current investigation, the therapeutic property of PZN was utilized for diagnostic rationale by tagging PZN directly with $^{99m}$Tc. The tagged moiety was characterized in terms of percent radiochemical purity, stability in normal saline at room temperature up to 24 h after reconstitution, in vitro stability in human serum at 37°C up to 24 h, in vitro binding with live and heat killed E. coli and biodistribution behavior in artificially E. coli infected animal model rats. The suitability of the $^{99m}$Tc-PZN as specific E. coli infection radio-tracer was scintigraphically evaluated and confirmed in artificially E. coli infected animal model rabbits.

### Experimental

#### Materials

Pazufloxacin (PZN) from Hunan Jiudian Pharmaceutical Co., Ltd. China, TLC from Merk (Germany), and all the other chemicals and solvents of analytical grade from Aldrich. RP-HPLC from Shimadzu, (Japan), well counter, scalor count rate meter from Ludlum (USA), Dose calibrator from Capintech (USA) and Gamma camera (GKS-1000, GEADE) from Nuclearmedizine system (Germany).

#### Methods

##### Preparation of Technetium-99m ($^{99m}$Tc) labeled Pazufloxacin (PZN)

Pazufloxacin (PZN) was labeled with Technetium-99m ($^{99m}$Tc) using the reported method [14]. Briefly, 1.0 mCi of sodium pertechnetate (Na$^{99m}$TcO$_4$) was mixed with 20 μg of stannous chloride. Thereafter, 1.0 mg of pure PZN was injected to the reaction vial through sterilized syringe with the removal of same amount of nitrogen from the reaction vial. The pH was adjusted to 5.5 with 0.01 mol/L of HCl, followed by filtration through millipore filters and incubation at room temperature. The overall process was repeated by rising the amount of stannous chloride from 20 to 200 μg (i.e. 20 μg rise in each repeating procedure until the amount of stannous chloride attains a level of 200 μg), Na$^{99m}$TcO$_4$ from 0.5 to 5.0 mCi (i.e. 0.5 mCi increase in each repeating procedure until the amount of Na$^{99m}$TcO$_4$ becomes 5.0 mCi) and amount of pure PZN from 1.0 to 10.0 mg (i.e. 1.0 mg increase in each repeating procedure).

##### Radiochemical Purity (RCP) yield

TLC method was employed for the determination of %RCP yield of the $^{99m}$Tc labeled PZN using dual mobile system. The developed strips were analyzed for %RCP yield using well counter.

##### In vitro stability in normal saline

High Performance Liquid Chromatography (HPLC) was used for the determination of in vitro stability in normal saline and characterization of the $^{99m}$Tc labeled PZN. In this experiment, HPLC (Shimadzu SCL-10 AVP system) connected with well counter was used. C-18 column was employed as stationary and triethylaminophosphate and methyl alcohol as the mobile phase. The flow rate (ml/min) was sustained for 20 min.

Further, the stability was examined in challenge assay by incubating equimolar solution of the $^{99m}$Tc labeled PZN with increasing concentrations of the cystein ($10^{-4}$, $10^{-3}$, $10^{-2}$, 0.1, 10,100 and 1000 mmol/l). The stability during incubation at 37°C was determined at different intervals by TLC followed by activity estimation using well counter.

##### Binding with E. coli

Binding behavior of the $^{99m}$Tc labeled PZN with E. coli (live and heat killed) was investigated using the reported method. Briefly, 0.5 mCi of the radiolabeled ($^{99m}$Tc-PZN) was mixed with 0.1 ml sodium phosphate buffer (Na-PB) followed by addition of 0.8 ml, $1 \times 10^8$ colony forming units (cfu) of the E. coli (live and heat killed) and 0.01 M acetic acid (50%, v/v). Thereafter, mixture was incubated at 4°C for 30 min, followed by centrifugation at 4000 rpm for 10 min. The percent binding was determined using well counter.

##### Biodistribution in animal model rat

Twenty healthy wister rats (weight, 180-210 gm) were used as experimental animals for determination of biodistribution behavior of the radiolabeled ($^{99m}$Tc-PZN). The selected animals were divided uniformly in two groups i.e (group A and B). Rats of group A and B rats were inflamed through intramuscular injection of pyrogen free oil into right leg of all rats. Thereafter, group A rats were infected by administering live strain of E. coli (live and heat killed) to the contra-lateral (left leg) of each rats and heat killed E. coli to the right leg of all rats. Thereafter, group A rats were infected by administering live strain of E. coli (0.1 mL containing $1 \times 10^8$ colony forming units (cfu) of the E. coli (live and heat killed) and 0.01 M acetic acid (50%, v/v)). Thereafter, mixture was incubated at 4°C for 30 min, followed by centrifugation at 4000 rpm for 10 min. The percent binding was determined using well counter.
lungs, stomach and intestines, kidney and urine using single well counter interface with scalar count rate meter.

**Scintigraphy**

Scintigraphic accuracy of the radiolabeled $^{99m}\text{Tc-PZN}$ was investigated using animal model rabbits (weight 4-5 kg). Healthy rabbits were anesthetized with 0.3 mL of ketamin i.m. followed by injecting 0.5 mL live strain of E. coli i.m. in to the right thigh. After 10 h, 1.0 mL pyrogen free oil was infused to the left leg of the same animal. Thereafter, 0.5 mL of the radiolabeled ($^{99m}\text{Tc-PZN}$) was administered to the animal model rabbit intravenously followed by whole body planer imaging at concentration value of the stannous chloride as 120 mg.

**Statistical analysis**

Results are expressed as %Injected dose/gram or ratios ± SEM and statistical analysis were executed using the student t -test

**Results and Discussion**

**Percent radiochemical purity (%RCP) yield**

The radio- HPLC chromatogram of $^{99m}\text{Tc-PZN}$ acquired at 30, 60, 90 and 120 min demonstrated analogous prototype signals as observed in case of PZN labeled either through $^{99m}\text{Tc≡N}$ [18] and or $^{99m}\text{Tc(CO)}_3$ [19] techniques. In case of $^{99m}\text{Tc-PZN}$, two broader different signals were observed one at 3.8 and second one at 10.9 min. The signal observed at 3.8 entail the unwanted side product consisting of free and reduced hydrolyzed $^{99m}\text{Tc}$; while the signal at 10.9 min, corresponds to main product i.e. labeled PZN, as shown in Figure 2.

The PZN labeled with $^{99m}\text{Tc}$ using different concentration of PZN, $^{99m}\text{Tc}$, stannous chloride at different pH showed different percent radiochemical purity yield. Further, it was observed that the labeled PZN primed by mixing 2 mg PZN, 120 μg stannous chloride, and 2.5 m Ci of sodium pertechnetate at pH 5.3, showed maximum radiochemical yield in percent at 30 min subsequent to labeling and remained intact more than 90% up to 240 min in normal saline. The percent radiochemical purity yield determined between at 1 to 240 min after labeling was 97.10 ± 0.28%, 98.25 ± 0.26%, 97.30 ± 0.36%, 96.00 ± 0.28%, 94.45 ± 0.24%, and 92.15 ± 0.32%, as given in Figure 3. However, the PZN labeled through $^{99m}\text{Tc≡N}$ [18] and or $^{99m}\text{Tc(CO)}_3$- [19] showed 98.40 ± 0.26% and 98.95 ± 0.28% maximum %RCP yield at 30 min after reconstitution and remain intact up to 90% up to 240 min. The percent radiochemical purity yield profile of labeled PZN, $^{99m}\text{Tc≡N-PZND}$ and $^{99m}\text{Tc(CO)}_3$-PZND showed close correlation.

Figure 4 showed the effect of the concentration of the PZN on the %RCP yield profile of the labeled PZN. It was observed that the %RCP yield profile of the labeled PZN increased initially by increasing the amount of the PZN in the reaction vial. However, this increase in the %RCP yield profile of the labeled PZN was noticed up to 2.5 mg of PZN and thereafter a gradual decrease was observed.

The effect of the activity of the $^{99m}\text{Tc}$ on the %RCP yield profile of the labeled PZN showed a similar trailing pattern. Initially the %RCP yield profile of the labeled PZN moves up by rising the concentration of the stannous pertechnetate. However, after adding 3.00 m Ci to the reaction vial the %RCP yield decrease. It is experiential that the labeled PZN showed maximum % RCP yield by adding 2.5 mg to the reaction vial.

The effect of different concentration of stannous chloride dehydrate on the %RCP yield of the labeled PZN have shown similar pattern as observed by varying the concentration of PZN and activity. Maximum %RCP yield was observed by adding 120 μg stannous chloride to the reaction vial.

Similarly the effect of pH on the %RCP yield of the labeled PZN is not different from the ones shown in Figure 4. It was observed that the labeled PZN showed higher %RCP yield at pH 3. The percent radiochemical purity yield profile of the labeled PZN goes down either by increasing or decreasing the pH from 3.

![Figure 2: HPLC radiochromatogram of $^{99m}\text{Tc-PZN}$.](image)

![Figure 3: Radiochemical Purity (%) of Technetium-99m labeled PZN up to 240 min after reconstitution at room temperature.](image)
In vitro stability in serum

The in vitro stability profile of the PZN labeled directly with $^{99m}$Tc at 37°C up to 16 h is given in Figure 5. The stability of labeled PZN in serum at 0, 2, 4, 6, 8, 10, 12, 14 and 16 h after reconstitution was 98.00 ± 0.30%, 93.30 ± 0.40%, 91.25 ± 0.35%, 88.55 ± 0.30%, 85.10 ± 0.35%, 83.45 ± 0.40%, 83.00 ± 0.36%, and 82.70 ± 0.50%. The free and colloidal $^{99m}$Tc based radioactivity up to 16.40% was calculated which is in permissible acceptable limit. However, the total decay observed after 16 h of incubation for $^{99m}$Tc≡N-PZND and $^{99m}$Tc(CO)$_3$-PZND were 15.95% and 13.35% [18,19]. The decay profile in terms of free and colloidal $^{99m}$Tc based radioactivity of labeled PZN, $^{99m}$Tc≡N-PZND and $^{99m}$Tc(CO)$_3$-PZND showed complete correlation and were in line with permissible acceptable limits.

Biodistribution in animal model rat

Table 1 showed % in vivo biodistribution behavior of the labeled PZN in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of the artificially infected model rats. Initially, the level of activity in blood was high which gradually went down up to 3.65 ± 0.18% and 4.25 ± 0.28%, in group A and B animal model rats respectively. No significant difference in the uptake of blood was seen in the PZN labeled either directly or through $[^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ or $[^{99m}$Tc≡N]$^{2+}$ technique as all showed similar trailing pattern initially the dose in blood was high which gradually went down, indicating its clearance from the circulatory system like a normal drug and the level of activity in liver, spleen, stomach and intestine of group A and B animals also disappeared gradually. Further the level of activity in liver of live and heat killed E. coli infected animal model rats (group A and B) showed no significant difference and also no significant difference was seen in the uptake, appearance and disappearance profile of labeled PZN, $^{99m}$Tc≡N-PZND [18] and $^{99m}$Tc(CO)$_3$-PZND [19]. Similarly, the appearance and disappearance profile of the labeled PZN, $^{99m}$Tc≡N-PZND [18] and $^{99m}$Tc(CO)$_3$-PZND [19] in spleen, stomach and intestine were also gone down with time and no substantial variation have been observed in the uptake live and heat killed E. coli infected rat. The level of activity uptake in kidneys of the labeled PZN, $^{99m}$Tc≡N-PZND [18] and $^{99m}$Tc(CO)$_3$-PZND [19] were up with time and no considerable distinction have been observed in the uptake live and heat killed E. coli animal model rats (group A and B).
In the infected muscle of the animal model rats artificially infected with live *E. coli* the uptake of the labeled PZN was low in the beginning but went up gradually. The uptake profile in the infected muscle of the animal model rats infected with live *E. coli* in case of labeled PZN injection were 6.35 ± 0.35%, 11.90 ± 0.21%, 14.75 ± 0.40%, and 13.80 ± 0.30% respectively, However, the level in the infected muscle of the animal model rats infected with heat killed *E. coli* in case of labeled PZN injection were 2.50 ± 0.10%, 3.00 ± 0.44%, 2.50 ± 0.15%, and 2.00 ± 0.24% respectively, which shows that the labeled PZN incorporated in the infected muscle (infected with live *E. coli*) in much higher concentration than the animal muscle infected with heat killed *E. coli*.

However no significant difference in the uptake profile of the inflamed muscle of the animal infected with live and heat killed *E. coli* were seen. Similarly the difference in uptake was negligible of the labeled PZN in the normal muscle of the animal model rats either infected with live or heat killed *E. coli*. The overall biodistribution of the labeled PZN, $^{99mTc}_{\equiv N}$-PZND [18], and $^{99mTc}_{\equiv (CO)\equiv}$-PZND [19] were found in close correlation with no significant difference with regards to the uptake in various organs of the animal model rats. Similar to $^{99mTc}_{\equiv N}$-PZND [18] and $^{99mTc}_{\equiv (CO)\equiv}$-PZND [19], the labeled PZN also moved out from the circulatory system and appeared in the urinary system confirming the normal route of excretion.

### Table 1: Biodistribution profile of the $^{99mTc}$ labeled PZN in artificially infected MWR.

| Organs/tissues (gm) | Distribution of the $^{99mTc}$-PZN in various organs |
|--------------------|------------------------------------------------------|
|                    | Live *E. coli*                                        |
|                    | Heat killed *E. coli*                                 |
| Infected muscle    | 6.35 ± 0.35, 11.90 ± 0.21, 14.75 ± 0.40, 13.80 ± 0.30 | 2.50 ± 0.10, 3.00 ± 0.44, 2.50 ± 0.15, 2.00 ± 0.24 |
| Inflamed muscle    | 3.00 ± 0.28, 3.50 ± 0.30, 3.00 ± 0.15, 3.00 ± 0.20   | 3.50 ± 0.22, 4.00 ± 0.24, 3.50 ± 0.20, 3.50 ± 0.18 |
| Normal muscle      | 2.00 ± 0.12, 3.00 ± 0.18, 2.50 ± 0.14, 2.00 ± 0.16   | 2.00 ± 0.14, 3.00 ± 0.20, 2.50 ± 0.18, 2.00 ± 0.22 |
| Blood              | 19.55 ± 0.17, 9.95 ± 0.18, 8.80 ± 0.20, 3.65 ± 0.18  | 20.10 ± 0.10, 9.40 ± 0.20, 7.95 ± 0.22, 4.25 ± 0.28 |
| Liver              | 18.15 ± 0.22, 13.00 ± 0.20, 11.10 ± 0.18, 4.20 ± 0.22 | 18.55 ± 0.15, 12.90 ± 0.10, 11.25 ± 0.22, 4.35 ± 0.18 |
| Spleen             | 9.15 ± 0.18, 7.10 ± 0.16, 6.15 ± 0.20, 4.00 ± 0.22   | 9.00 ± 0.22, 7.55 ± 0.28, 6.00 ± 0.16, 4.20 ± 0.26 |
| Kidney             | 10.25 ± 0.18, 16.00 ± 0.18, 19.50 ± 0.18, 22.55 ± 0.20 | 10.55 ± 0.20, 17.95 ± 0.26, 20.50 ± 0.24, 23.15 ± 0.18 |
| Stomach & intestines| 8.65 ± 0.18, 7.20 ± 0.20, 5.45 ± 0.12, 3.70 ± 0.20   | 8.80 ± 0.26, 7.35 ± 0.14, 5.70 ± 0.22, 4.00 ± 0.10 |

### Scintigraphy

Scintigraphically, it was observed that the labeled PZN showed initially normal activity uptake in the whole body of the animal model rat as shown in Figure 7.

![Figure 7: Scintigraphic image of artificially infected animal model rabbit.](image-url)

However, after few minutes, the activity accumulated in the infected muscle more than the inflamed and normal muscle. The level of the labeled PZN was much lower in kidneys in the begging but it went up and reached to a maximum level after 120 m. The scintigraphic visualization of artificially infectious sites validated the targeted biodistribution behavior of the labeled PZN.

### Conclusion

In this work PZN was labeled directly with Technetium-99m ($^{99mTc}$) and compared with the existing $^{99mTc}_{\equiv N}$-Pazufloxacin dithiocarbamate ($^{99mTc}_{\equiv (CO)\equiv}$-PZND) and $^{99mTc}_{\equiv (CO)\equiv}$-Pazufloxacin dithiocarbamate ($^{99mTc}_{\equiv (CO)\equiv}$-PZND) with the aim to provide an alternative radio-diagnostic agent for the deep tissue infection imaging (caused by *E. coli*) in its early stages. Based on the high radiochemical purity yield, stability in serum, in vitro binding with live *E. coli*, biodistribution in artificially infected animal model rats and scintigraphic assessment the labeled PZN is recommended for the diagnosis of deep tissue infection caused by *E. coli*.

### References

1. Litzler PY, Manrique A, Etienne M, Salles A, Edet-Sanson A, et al. (2010) Leukocyte SPECT/CT for detecting infection of left-ventricular-assist devices: preliminary results. J Nucl Med 51: 1044-1048.
2. Nanni C, Errani C, Boriani L, Fantini L, Ambrosini V, et al. (2010) 68Ga-citrate PET/CT for evaluating patients with infections of the bone: preliminary results. J Nucl Med 51: 1932-1936.
3. Filippi L, Uccio L, Giurato L, Schillaci O (2009) Diabetic foot infection: usefulness of SPECT/CT for 99mTc-HMPAO-labeled leukocyte imaging. J Nucl Med 50: 1042-1046.
4. Belloli S, Briosci A, Politi LS, Ronchetti F, Calderoni S, et al. (2013) Characterization of biological features of a rat F98 GBM model: a PET-MRI study with [18F]FAZA and [18F]FDG. Nucl Med Biol 40: 831-840.

5. Mirbolooki MR, Upadhyay SK, Constantinescu CC, Pan ML, Mukherjee J (2014) Adrenergic pathway activation enhances brown adipose tissue metabolism: a [18F]FDG PET/CT study in mice. Nucl Med Biol 41: 10-16.

6. Wang Y, Chen L, Liu X, Cheng D, Liu G, et al. (2013) Detection of Aspergillus fumigatus pulmonary fungal infections in mice with (99m)Tc-labeled MORF oligomers targeting ribosomal RNA. Nucl Med Biol 40: 89-96.

7. Liu X, Cheng D, Gray BD, Wang Y, Akalin A, et al. (2012) Radiolabeled Zn-DPA as a potential infection imaging agent. Nucl Med Biol 39: 709-714.

8. van der Bruggen W, Bleeker-Rovers CP, Boerman OC, Gotthardt M, Oyen WJ (2010) PET and SPECT in osteomyelitis and prosthetic bone and joint infections: a systematic review. Semin Nucl Med 40: 3-15.

9. Andrade Martins Pd, Osso JA Jr (2013) Thermal diffusion of 67Ga from irradiated Zn targets. Appl Radiat Isot 82: 279-282.

10. Liu Z, wyffels L, Barber C, Hui MM, Woolfenden JM (2011) A (99m)Tc-labeled dual-domain cytokine ligand for imaging of inflammation. Nucl Med Biol 38: 795-805.

11. Chattopadhyay S, Ghosh M, Sett S, Das MK, Chandra S, et al. (2012) Preparation and evaluation of 99mTc-cefuroxime, a potential infection specific imaging agent: a reliable thin layer chromatographic system to delineate impurities from the 99mTc-antibiotic. Appl Radiat Isot 70: 2384-2387.

12. Chattopadhyay S, Saha Das S, Chandra S, De K, Mishra M, et al. (2010) Synthesis and Evaluation of (99m)Tc-moxifloxacin, a Potential Infection Specific Imaging Agent. Appl Radiat Isot 68: 314-316.

13. Watabe S, Yokoyama Y, Nakazawa K, Shinozaki K, Hiraoka R, et al. (2010) Simultaneous measurement of paxloflaxacin, ciprofloxacin, and levofloxacin in human serum by high-performance liquid chromatography with fluorescence detection. J Chromatogr B Analyt Technol Biomed Life Sci 878: 1555-1561.

14. Shah SQ, Khan MR, Ali SM (2011) Radiosynthesis of (99m)Tc(NO3)3-Clinafloxacin Dithiocarbamate and Its Biological Evaluation as a Potential Staphylococcus aureus Infection Radiotracer. Nucl Med Mol Imaging 45: 248-254.

15. Benitez A, Roca M, Martin-Comin J (2006) Labeling of antibodies for infection diagnosis. Q J Nucl Med Mol Imaging 50: 147-152.

16. Shah SQ, Khan AU, Khan MR (2010) Radiosynthesis and biodistribution of (99m)Tc-rifampicin: a novel radiotracer for in-vivo infection imaging. Appl Radiat Isot 68: 2255-2260.

17. Kaul A, Hazari PP, Rawat H, Singh B, Kalawat TC, et al. (2013) Preliminary evaluation of technetium-99m-labeled ceftriaxone: infection imaging agent for the clinical diagnosis of orthopedic infection. Int J Infect Dis 17: e263-270.

18. Shah SQ, Khan MR (2015) Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry. Radioanal. Nucl. Chem 201: 190-195.

19. Welling MM, Paulusma-Annema A, Balter HS, Pauwels EK, Nibbering PH (2000) Technetium-99m labelled antimicrobial peptides discriminate between bacterial infections and sterile inflammations. Eur J Nucl Med 27: 292-301.