# $^{99m}$Tc-Labeled Ceftazidime and Biological Evaluation in Experimental Animals for Detection of Bacterial Infection

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**Abstract** Nuclear medicine is a powerful diagnostic technique able to detect inflammatory foci in human disease by using radiolabeled antimicrobial agents. Our goal was labeling of ceftazidime with technetiun-99m using stannous chloride dehydrate as a reducing agent. Under optimum conditions, the labeling yield of $^{99m}$Tc-ceftazidime complex, was $86.5 \pm 1.3\%$, the complex was stable for 2 hours after labeling. Biodistribution studies in mice were carried out in experimentally induced infection in the left thigh using *staphylococcus aureus*. The ratio of bacterial infected thigh/contralateral thigh was then evaluated. The abscess-to-muscle ratio for $^{99m}$Tc-ceftazidime was $5.06\pm0.8\%$ while that for commercially available $^{99m}$Tc-Ciprofloxacin was $3.8\pm0.5\%$ under the same experimental conditions, indicated that $^{99m}$Tc-ceftazidime could be used for infection imaging.

**Keywords** Ceftazidime, Technetium-99m, Labeling, Inflammation

## 1. Introduction

Radiopharmaceuticals play a major role in providing the best possible solutions for inflammation diagnosis and treatment. In the last few decades, a large number of radiopharmaceuticals are developed for imaging of infection and inflammation. Radiolabeled compounds are injected intravenously and accumulate in the inflammatory lesion due to locally changed physiological condition after administration, the excretion occurs by glomerular filtration and tubular secretion within 24 hour being almost totally eliminated [1].

The conventional imaging techniques such as radiology, computerized tomography (CT) and nuclear magnetic resonance (NMR) are not capable of differentiating between inflammatory and infectious process. In addition, these techniques are based on important anatomic alterations and the possibility of a precocious diagnosis is limited. Contrary to conventional imaging techniques, imaging scintilography is based on physiological and biochemical alteration resulting from inflammatory and infectious process, and then further progress has been made on the use of labeled antimicrobial agents as selective markers for diagnosis of bacterial and fungal infections [2].

The theoretical advantage of using antimicrobial agents as the localizing agent for infective foci is the selective toxicity of the compound for microbial rather than human targets. Such agents should therefore be able to distinguish between inflammations due to infection with microbial pathogens and inflammation due to injury or immune activity i.e. autoimmune disease where microbes are not involved. The antimicrobial agents have the potential to influence clinical decisions in the management of complicated conditions such as fever of unknown origin or occult infection [3].

The radiopharmaceuticals used for scintilographic detection include $^{67}$Ga-citrate, $^{99m}$Tc or $^{111}$In-labeled leukocytes, $^{99m}$Tc or $^{111}$In-labeled human polyclonal immunoglobin, $^{99m}$Tc labeled antibiotic. There are several reasons why imaging of infection and inflammation becomes increasingly important in the next decade. The population is ageing; the application of implants and transplants is increasing. The number of immune compromised patients is growing, mainly because of frequent use of chemotherapeutic agents leading to neutropenia. Furthermore, the increased use of antibiotics leads to insensitivity for some of these pharmaceuticals [4, 5].

The first antibiotic was ciprofloxacin radiolabeled with $^{99m}$Tc, which is supposed to bind DNA-gyrase and topoisomerase Iv of bacteria, as does unlabeled ciprofloxacin [6]. However, $^{99m}$Tc-ciprofloxacin preparation has some disadvantages related to radiochemical purity (81±4%) and stability, so other antimicrobial agents such as levofloxacin, pefloxacin, norfloxacin and cefoperazone were labeled with $^{99m}$Tc to be used for imaging sites of infection and overcome the drawback of $^{99m}$Tc-ciprofloxacin [7, 8].

Ceftazidime (fortum) is one from the third generation cephalosporin has a wide spectrum of activity to the

beta-lactamases, binds to the bacterial wall inhibits the synthesis of peptidoglycan and therefore inhibits the synthesis of bacterial wall which drives to bacterial death. Therapeutic actions: ceftazidime has a bactericidal effect; action depends on ability to reach and bind penicillin-binding proteins located in bacterial cytoplasmic membranes. Cephalosporins inhibit bacterial septum and cell wall synthesis, probably by acylation of membrane-bound transpeptidase enzymes. This prevents cross-linkage of peptidoglycan chains, which is necessary for bacterial cell wall strength and rigidity. Also, cell division and growth are inhibited, and elongation of susceptible bacteria and lysis frequently occur. Rapidly dividing bacteria are those most susceptible to the action of cephalosporins [9, 10].

The aim of this work was labeling of ceftazidime with technetiun-99m, study the labeling conditions and biological distribution of 99mTc-ceftazidime in inflammation bearing animals.

## 1.1. Materials

All the chemical reagents were of analytical grade. Bidistilled water was used for solution preparation, with nitrogen purging in the case of labeling studies. Ceftazidimewas purchases from Glaxo Smith Kline, Pharmaceutical Company, Cairo., Egypt. Ceftazidime structure is shown in Figure (1). Albino Swiss mice weighing, 25-40 g were used for studying biodistribution [11].
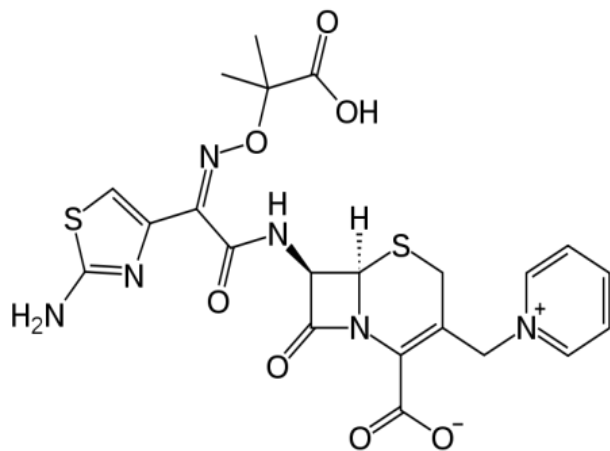


**Figure 1.**   Structure of Ceftazidime

## 1.2. Methods

### 1.2.1. Labeling Procedure

Accurately weighed 1 mg Ceftazidime was transferred to apenicillin then the vial was evacuated with nitrogen gas. Exactly 100 µl of $SnCl_2.2H_2O$ stock solution (10µgSn) was added, then the volume of the reaction mixture was completed to one ml by $N_2$-purged distilled water, added 0.5ml buffer pH 10. Finallyadded 0.5 ml of freshly eluted $Na^{99m}TcO_4^-$ (~500MBq) to the above mixture. The mixture was shaken and allows reacting at room temperature for sufficient time required to complete the reaction.

### 1.2.2. Radiochemical Purity

#### 1.2.2.1. TLC Analysis

The labeling yield and radiochemical purity were determined by layer chromatographic. The reaction product was spotted on silica gel TLC-SG strips (Sigma Chemical Company, USA) (10 x 1.5 $cm^2$ sheets). And developed in acetone and mixture, ethanol: water: ammonium hydroxide (2:5:1) as the mobile phase. After developing, the strips were dried, cut into 0.5 cm pieces and separately counted using the NaI(Tl) scintillation counter todetermine the ratio of hydrolyzed 99mTc, free 99mTcO4− and 99mTc-complex each experiment was repeated three times [12].

#### 1.2.2.2. Electrophoresis Analysis

It was further confirmed by electrophoresis (100 V, 30 mA, 20 W) by adding about 5 µl spot from the reaction mixture before filtration and after filtration to eliminate colloid impurities using Millipore filter of 0.22 µm size on each cellulose acetate strip, 2 cm width and 46 cm length which divided with a non-pointed pencil into fragments from zero point into 20 fragments, these strips were moistened with 0.9% N Saline solution as an electrolytes source, then placed in the chamber, after 2 hours developed strips were removed, dried and cut into one cm each fragment, then counted using gamma counter, percentage of radiochemical yield was estimated as the radioactivity of the labeled compounds to the total activity multiplied by 100 [13, 14, 15].

#### 1.2.2.3. Stability of 99mTc-Ceftazidime

Stability of 99mTc-Ceftazidimewas studied at different time intervals up to 24 h post labeling by TLC for determination of the percent yield of 99mTc-complex, reduced hydrolyzed technetium and free Pertechnetate.

#### 1.2.2.4. Induction of Infectious Foci

A single clinical isolation of *Staphylococcus aureus* from biological samples was used to produce focal infection. Individual colonies containing $10^5$-$10^6$ organisms were diluted with saline in order to obtain a turbid suspension. Groups of five mice weighing 25-40 g were intramuscularly injected with 200 µl of the suspension in the left lateral thigh muscle. Then, the mice were left for 24 h to get a gross swelling in the infected thigh [16].

#### 1.2.2.5. Induction of Non-infected Inflammation

Sterile inflammation was induced by injecting 200 µl of turpentine oil, sterilized by autoclaving at 121ºC for 20 min, intramuscularly in the left lateral thigh muscle of the mice. Two days later, swelling appeared [17].

#### 1.2.2.6. Induction of Heat Killed Staphylococcus Aureus Non Infected Inflammation

Sterile inflammation was induced by injecting 200 µl of heat killed ***Staphylococcus aureus***, sterilized by autoclaving

at 121ºC for 20 min, intramuscularly in the left lateral thigh muscle of the mice. Two days later, swelling appeared [18].

### 1.2.2.7. Biochemical Investigations

The blood samples were collected directly from each mice into a sterile tube, and then was centrifuged for 10 minutes to obtain the serum sample, which was stored frozen at -20ºC until analyzed. Some biochemical parameters were performed before and after inflammation induction as a markers for inflammation such as: $C_3$, $C_4$, LDH, CPK, ALT, AST, Albumin, TotalProtein, Urea, Creatinine [19].

### 1.2.2.8. Biodistribution Studies

Biodistribution of the sterile $^{99m}$Tc-ceftazidime complex was evaluated in male Albino Swiss mice weighing 25-40 g. For the quantitative determination of organ distribution, five mice were used for each experiment and 0.1 ml of about 37 MBq of $^{99m}$Tc-Ceftazidime solution was injected into the tail vein of mice after 24 h of bacterial induction. The mice were anesthetized with chloroform then sacrificed. Samples of infected muscle contralateral, normal muscle, blood, bone, liver, kidney, stomach, intestine, spleen, lung, heart, and urinary bladder were weighed, and the radioactivity was measured using a gamma counter. The results were expressed as the percent uptake of injected dose per gram of tissue (ID/g) Blood, bone and muscles were assumed to be 7, 10, 40%, respectively, of total body weight. Corrections were made for background radiation and physical decay

during experiment. Both target/non target thighs were dissected and counted. Target/non-target thigh radioactivity ratio was also determined [20].

### 1.2.3. Statistical Analysis

Data were expressed as mean ±SD. Statistical analysis was performed using analysis of variance (ANOVA) with multiple comparison tests. A probability of less than 0.05 was considered to be significant.

## 2. Results and Discussion

Radiochemical purity and stability of $^{99m}$Tc-ceftazidime complex were assessed by thin layer chromatographic method. In thin layer chromatography using acetone as the solvent (about 15-30 min required for developing solvent). Free pertechnetate moves with the solvent front ($R_f$=1), while $^{99m}$Tc-ceftazidime complex and reduced hydrolyzed technetium remained at the origin. Reduced hydrolyzed technetium was determined by using mixture ethanol: water: ammonium hydroxide mixture (2:5:1) as the mobile phase where reduced hydrolyzed technetium remains at the origin ($R_f = 0$) while other species migrate with the solvent front ($R_f = 1$). The radiochemical purity was determined by subtracting the sum of the percent of colloid and free Pertechnetate from 100%. The radiochemical yield is the mean value of three experiments [21].
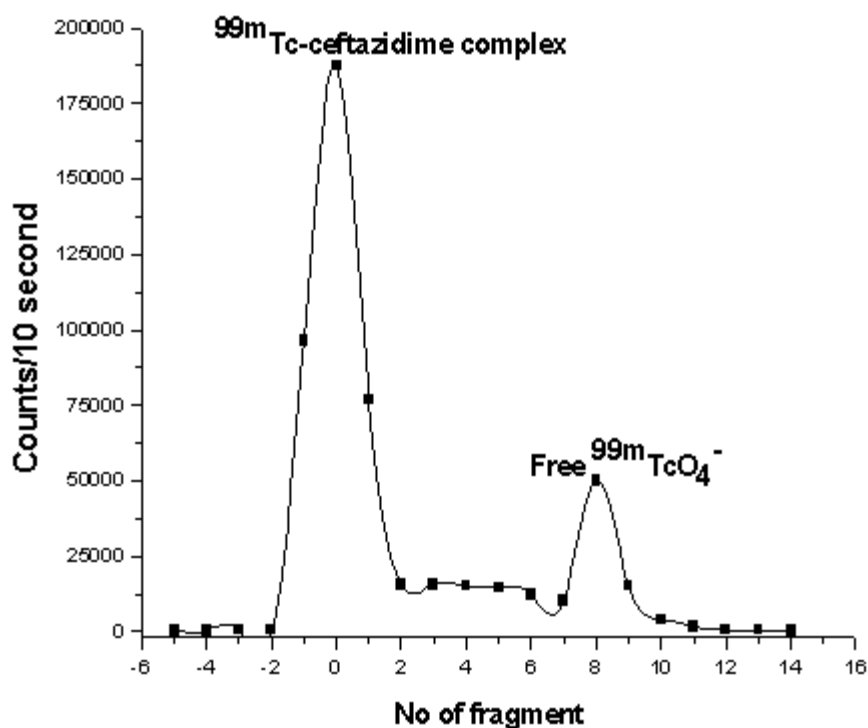


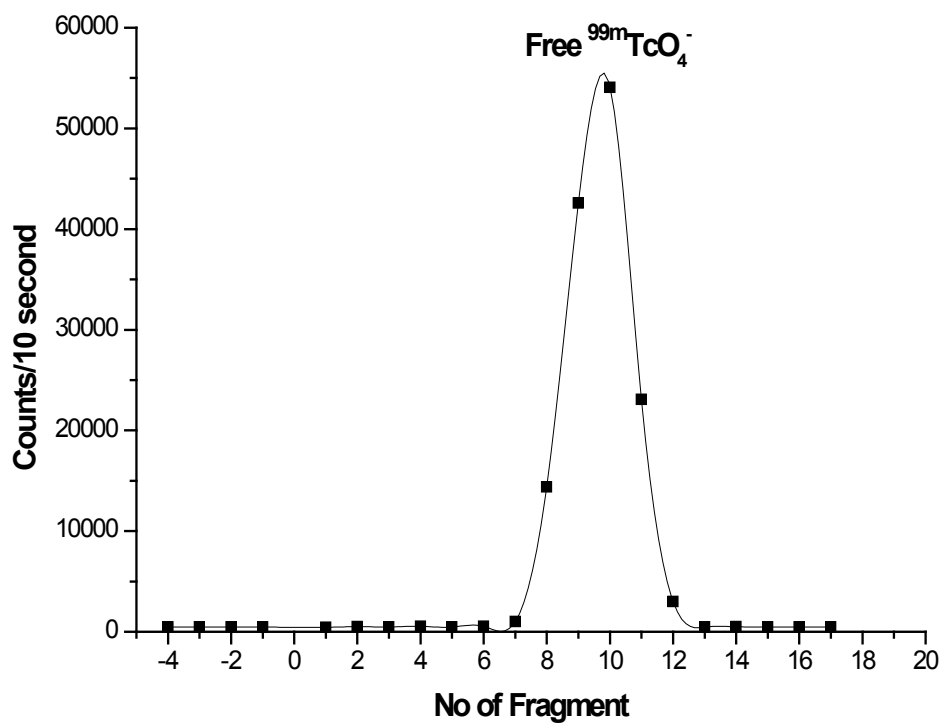**Figure 2.** Electrophoresis radiochromatogram of $^{99m}$Tc-ceftazidime

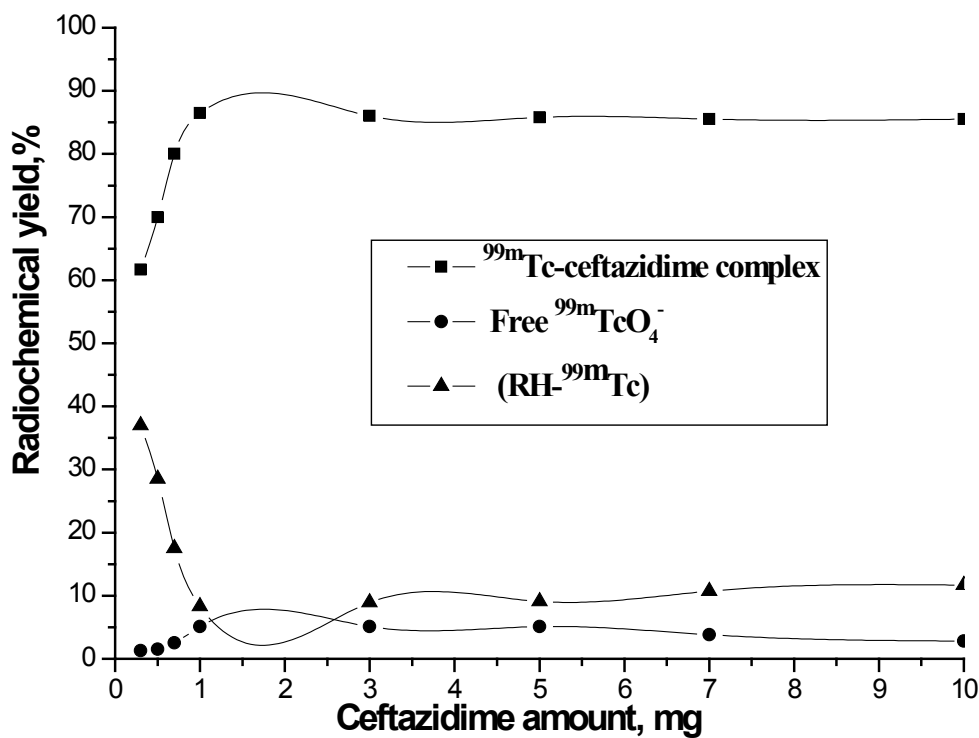**Figure 3.**   Electrophoresis radiochromatogram of free [99m]TcO4^{-}



**Figure 4.**   Variation of the radiochemical yield of [99m]Tc- ceftazidime as a function of ceftazidime amount; reaction conditions: x mg of ceftazidime, dissolved in1 ml D.D $H_2O$, 10 µg of $SnCl_2.2H_2O$, 0.5 ml (~500MBq) of [99m]TcO4 , the reaction mixture was kept at room temperature for 30 min

On the other hand, the electrophoresis purification of ^{99m}Tc-ceftazidime complex was presented in Figure (2) and showed two peaks, one at fraction No. zero, which corresponds to [99m]Tc- ceftazidime complex (86%) while the second peak was collected at fraction No. 8, which corresponds to free [99m]TcO$_4^-$ (14%), while the colloid remove by filtration using milipore filter. While Figure (3) represent the electrophoresis for free Pertechnetate only which forms one peak started from fragment No. 8 [22, 23].

The chemical structure of ceftazidime is presented in figure (1). The various complexes of ^{99m}Tc may be formed by interactions between electron donor such as oxygen, nitrogen and sulfer. Although the exact complex structure is not known, all cephalosporines coordinate with ^{99m}Tc due to the existence of electron donor atoms in their structure [24, 25, 26].

### 2.1. Factors Affecting the Labeling Yield

#### 2.1.1. Effect of Substrate Amount

Figure 4 shows that, the radiochemical yield of ^{99m}Tc-ceftazidime complex increased from 61.7% at 0.3 mg of ceftazidime to 86.5% at 1 mg ceftazidime by increasing the amount of ceftazidime. When the ceftazidime amount increased above 1mg the labeling yield slightly decreased, then above 3 mg of ceftazidime, the labeling yield remained stable. So, the optimum amount of ceftazidime was 1 mg, below This amount the yield decrease due to the fact that at low ceftazidime amount, SnCl$_2$.2H$_2$O is greater than that of ceftazidime, and is easily converted to colloid as the percentage of colloid was 37% using 0.3 mg ceftazidime.

#### 2.1.2. Effect of SnCl$_2$. 2H$_2$O Amount

As shown in Figure. 5, at 1 µg of SnCl$_2$.2H$_2$O the radiochemical yield of ^{99m}Tc-ceftazidime complex was (76%) and this low yield due to SnCl$_2$.2H$_2$Oamount was insufficient to reduce all pertechnetate present in the reaction mixture. It was observed that the yield increased by increasing the amount of SnCl$_2$.2H$_2$O till reached 86.5% at 10µg SnCl$_2$.2H$_2$O due to sufficient amount of SnCl$_2$.2H$_2$O to reduce all pertechnetate present in the reaction mixture. By increasing the amount of SnCl$_2$.2H$_2$O the labeling yield decreased gradually till reached to (60.1%) at 200 µg SnCl$_2$.2H$_2$O due to colloid formation. This may be due to the fact that most of the ligand molecules were consumed in the formation of complexes, so the pertechnetate is reduced to insoluble technetium (IV) TcO$_2$.xH$_2$O in the absence of ligand [20] or due to the fact that the excess amount of stannous chloride leads to the formation of stannous hydroxide colloid Sn(OH) [27].



**Figure 5.** Variation of the radiochemical yield of [99m]Tc- ceftazidimeas a function of SnCl$_2$.2H$_2$O amount; reaction conditions: 1mg of ceftazidime, dissolved in1 ml D.D H$_2$O, x µg of SnCl$_2$.2H$_2$O, 0.5 ml (~500MBq) of [99m]TcO4$^-$, the reaction mixture was kept at room temperature for 30 min

**Figure 6.**  Effect of pH of the reaction mixture on the radiochemical yield of [99m]Tc- ceftazidime; reaction conditions: 1 mg of ceftazidime, dissolved in1 ml pH x, 10 µg of $SnCl_2.2H_2O$, 0.5 ml (~500MBq) of [99m]$TcO_4$, the reaction mixture was kept at room temperature for 30 min
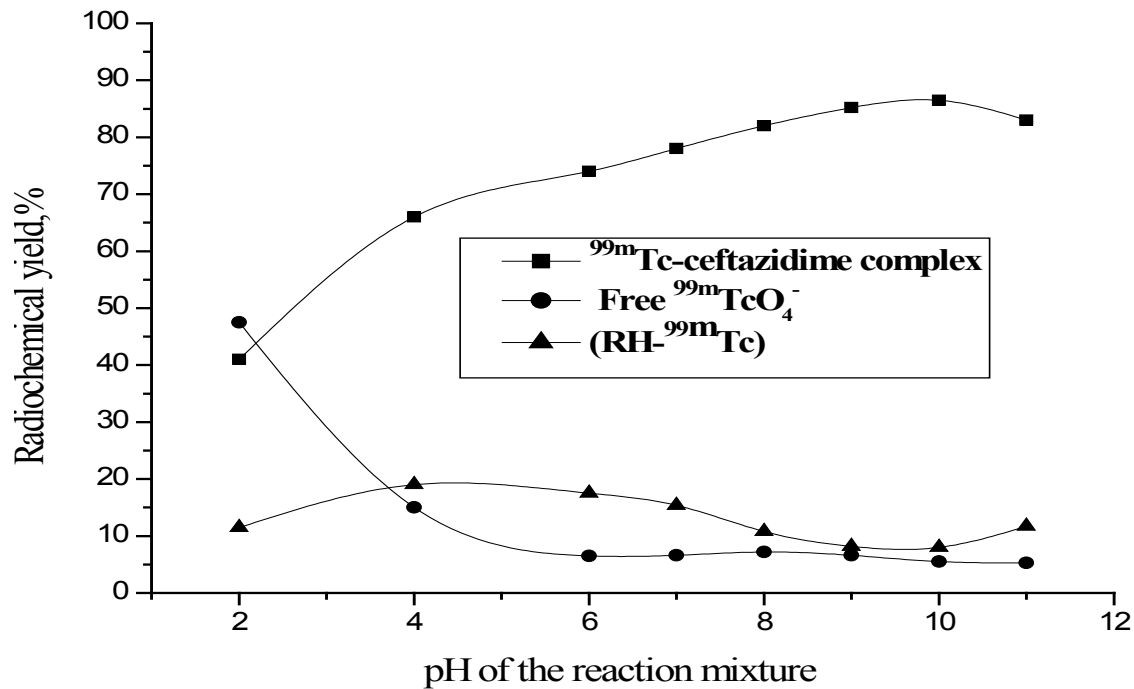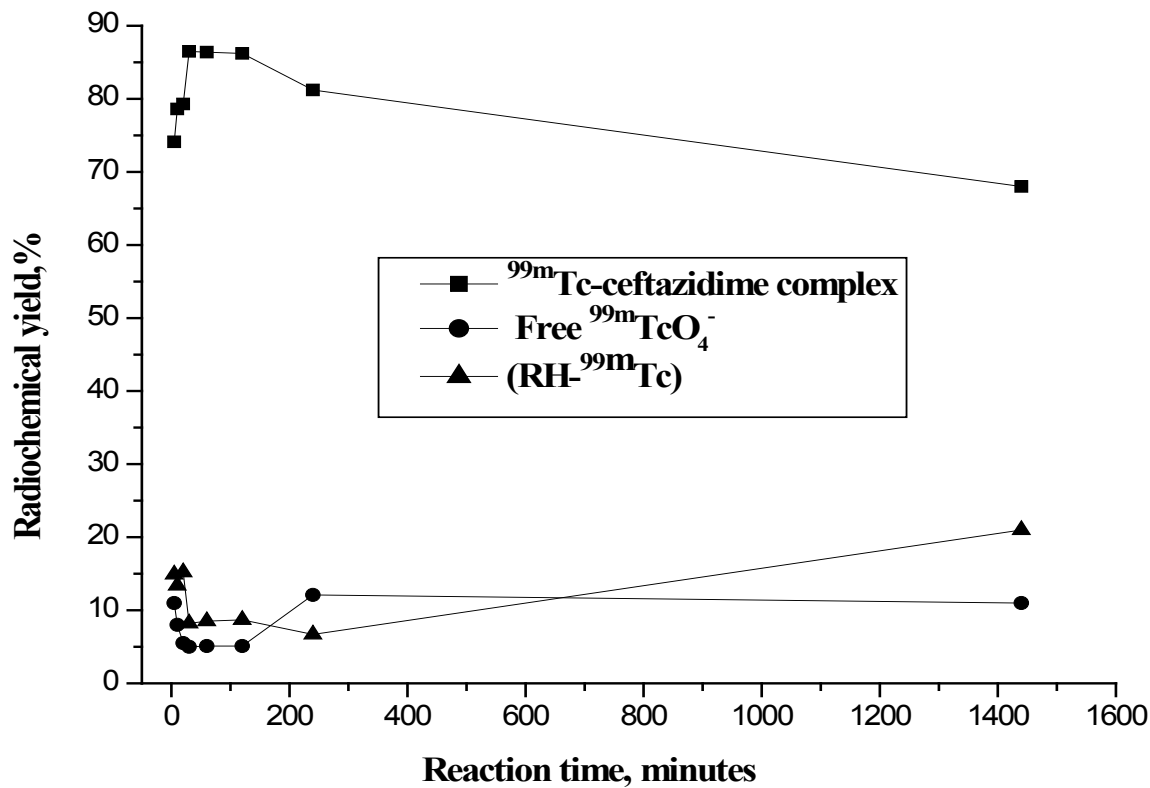


**Figure 7.**  Effect of reaction time on the radiochemical yield of [99m]Tc- ceftazidime; reaction conditions: 1 mg of ceftazidime, dissolved in1 ml pH 10, 10 µg of $SnCl_2.2H_2O$, 0.5 ml (~500MBq) of [99m]$TcO_4^-$, the reaction mixture was kept at room temperature at different timeintervals

### 2.1.3. Effect of pH of the Reaction Mixture

As shown in Figure. 6, at pH 2, the labeling yield of $^{99m}$Tc-ceftazidime complex was 41% and the yield increased with increasing the pH of the reaction mixture to a maximum yield of 86.5% at pH 10. At pH below or above the optimum pH, the radiochemical purity is significantly decreased by forming reduced hydrolyzed technetium-99m which is the main radiochemical impurity.

### 2.1.4. Effect of Reaction Time and Stability

As shown in Figure 7, the labeling of ceftazidime with technetium-99m was done at room temperature (25ºC) and carried out at different intervals of time. The labeling reaction was complete after 30 min with a radiochemical yield of 86.5%. The formed complex was stable for a time up to 2 h, after that the yield decreased till reached 68% after 24 h from the post labeling due to colloid formation.

### 2.1.5. Effect of Reaction Temperature

As shown in Fig. 8, the radiochemical yield of $^{99m}$Tc-ceftazidime complex was maximum (86.5%) at room temperature 25ºC, then decreased gradually to reached (70%) at 95ºC.

## 2.2. Biodistribution in Animals

### 2.2.1. Biochemical Investigations

The results in table 1 showed increasing in the biochemical parameters results after inflammation with bacteria induction in animals than before inflammation induction, liver enzymes (aspartate aminotransferase AST, alanine aminotransferase ALT), kidney function tests (serum creatinine, blood urea nitrogen and total protein) were increased in state of inflammation than in normal mice, while serum albumin was decreased in state of inflammation [23]. There were high increase in the muscle markers enzymes Lactate Dehydrogenase (LDH), Creatinine Phosphokinase (CPK) also high increase in amount of complement 3, complement 4, c-reactive protein (CRP) in state of inflammation than in normal mice, which play an important role in host defense mechanisms against infection, The human immune system is notable for its ability to combat infectious microorganism by cliciting inflammatory responses such as acute phase protein (CRP) which elevated markedly in association with infection and inflammation [28, 29].
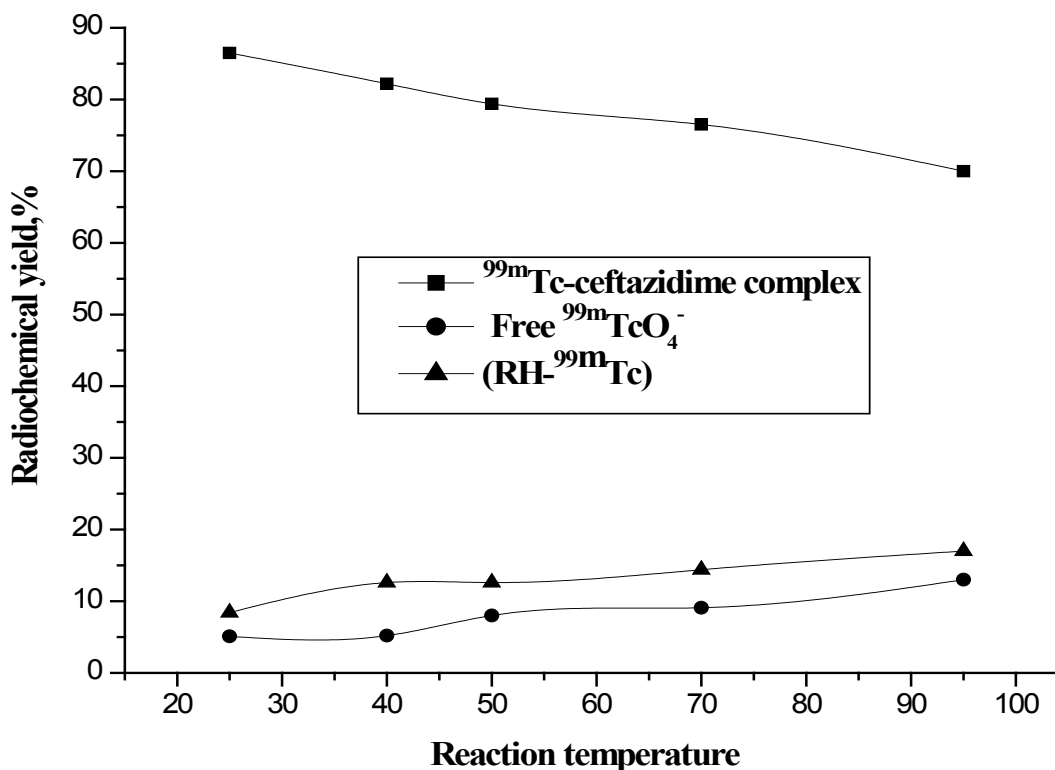


**Figure 8.** Effect of temperature on the radiochemical yield of $^{99m}$Tc- ceftazidime; reaction conditions: 1 mg of ceftazidime, dissolved in1 ml pH 10, 10 μg of SnCl$_2$.2H$_2$O, 0.5 ml (~500MBq) of $^{99m}$TcO$_4^-$, the reaction mixture was kept at different temperature for 30 minute
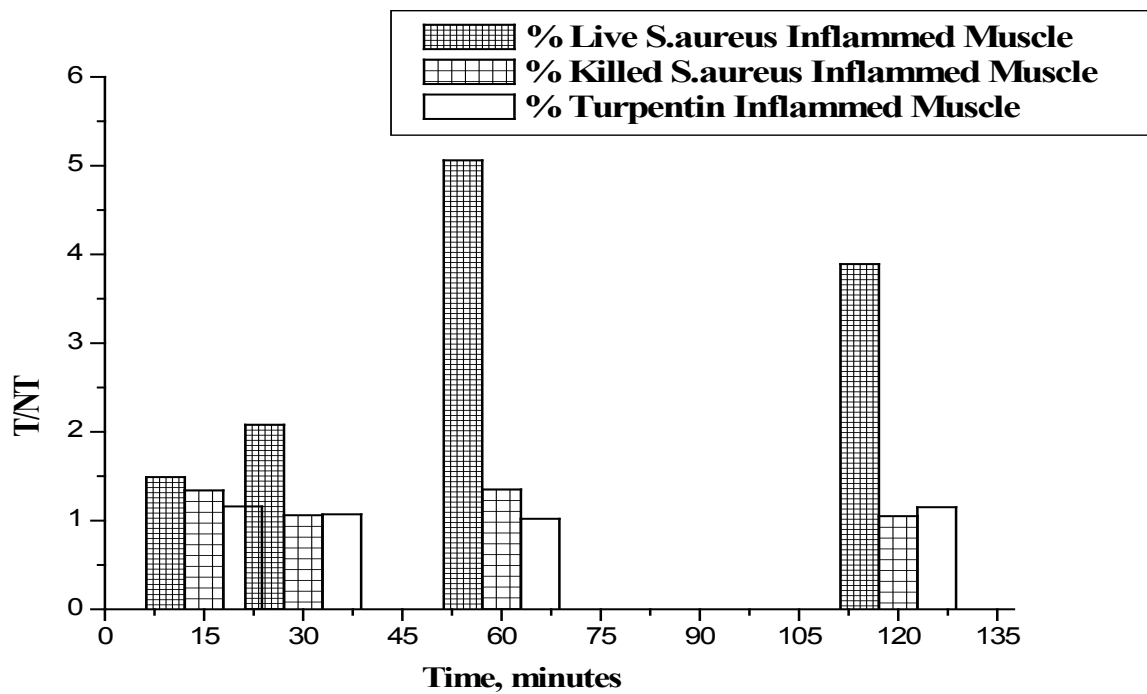
**Figure 9.**   T/NT of live S.aureus, Killed S.aureus and turpentine oil at 15, 30, 60, 120 min post injection $^{99m}$Tc-Ceftazidim

**Table 1.**   Results of inflammation markers, liver and kidney function test before and after livebacteria induction in mice

| Biochemical Parameters | Normal Mice | Inflamed Mice |
|---|---|---|
| C3(mg/dl) | 48.9±4.0 | *156±3.8 |
| C4(mg/dl) | 5.6±1.3 | *54±1.2 |
| LDH(u/l) | 560±151 | *6126±586 |
| CPK(u/l) | 320±46 | *7893±120 |
| CRP(mg/l) | Negative (<6±0.0) | Positive *24±6.0 |
| GPT(u/l) | 35±5.8 | 73±5.1 |
| GOT(u/l) | 57±4.1 | 156±15.2 |
| Total protein(g/dl) | 7.1±0.2 | 8.1±0.2 |
| Serum Albumin(g/dl) | 3.6±0.35 | 2.5±0.18 |
| Serum Creatinine(mg/dl) | 0.8±0.2 | 1.8±0.2 |
| Blood Urea(mg/dl) | 34±5.5 | 86±7.6 |

### 2.2.2. Bidistribution

The uptake of the $^{99m}$Tc-ceftazidime complex in different organs of the animals infected with turpentine oil, s. aureus and killed s.aureus was given in table (2a,b,c), respectively. $^{99m}$Tc-ceftazidime was removed from the circulation mainly through the urinary pathway after 2 h post injection of the tracer. In the turpentine oil inflamed mice, a significant large amount of $^{99m}$Tc-ceftazidime activity was observed in the liver (12.3%) after 30 min post injection. After 2 h of tracer administration, the major part of activity of $^{99m}$Tc-ceftazidime was found in the kidney (8.5±1.7), liver (13.6±0.1) and spleen (5.8±0.1) as shown in table 2 a. In contrast, in s.aureus inflamed mice, the liver uptake decreased than that in turpentine oil from 2.97% after 30 min till reaching 4.27% after 2 h as shown in table 2b. The uptake

of $^{99m}$Tc-ceftazidime was significantly low in turpentine, killed s.aureus infected group of animals (Aseptic inflammation) as compared to infected group with living bacteria (abcess). These data indicate that the distribution throughout the body and uptake in the inflamed area was observed within 60 min after intravenous injection of the tracer.

**Table 2a.**   Biodistribution of $^{99m}$Tc-ceftazidime in body organs inflamed mice for turpentine oil at 15, 30, 60 and 120 min post injection

| Organ Time (min) | Turpentin Oil | | | |
|---|---|---|---|---|
| | 15 | 30 | 60 | 120 |
| **Blood** | 11.2±0.04 | 17.6±0.2 | 10.9±0.3 | 4.2±0.2 |
| **Inflammed Muscle** | **2.61±0.1** | **1.4±0.1** | **0.44±0.2** | **0.4±0.2** |
| **Normal Muscle** | **2.24±0.04** | **1.3±0.03** | **0.43±0.1** | **0.35±0.1** |
| **Bone** | 4.0±0.12 | 2.37±1.0 | 2.38±1.0 | 0.98±1.1 |
| **Stomach** | 0.42±0.1 | 0.77±0.03 | 1.61±0.2 | 0.8±0.1 |
| **Intestine** | 1.72±0.0 | 2.03±0.1 | 1.43±0.1 | 2.52±0.2 |
| **Kidneys** | 15.3±0.2 | 14.3±0.2 | 15.6±0.3 | 8.5±1.7 |
| **Liver** | 4.0±0.1 | 12.3±0.1 | 12.5±0.1 | 13.6±0.1 |
| **Spleen** | 1.4±0.1 | 8.5±0.1 | 5.1±0.0 | 5.8±0.1 |
| **Lung** | 4.6±0.0 | 4.02±0.0 | 2.06±0.1 | 3.9±0.1 |
| **Heart** | 4.9±0.1 | 6.5±0.1 | 2.4±0.1 | 3.3±0.1 |
| **Urine** | 10.4±0.1 | 33±1.2 | 19.5±6.2 | 16.9±3.2 |
| **T/NT** | **1.16±0.1** | **1.07±0.1** | **1.02±0.1** | **1.15±0.3** |

As shown in Fig (9), mice with infectious lesion injected with $^{99m}$Tc-ceftazidime showed a mean abscess-to-muscle (target-to-non target, T/NT) for live s.aureus, killed s.aureus

and turpentine oil at 15, 30, 60 and 120 min post injection [99m]Tc- ceftazidime, respectively. The retention was specific since the abscess uptake of [99m]Tc- ceftazidimeremained high (T/NT= 5.06) when compared with the uptake of septic foci at 1 h post injection, the high T/NT ratio for lives. aureus model in comparison to the turpentine oil and killed s.aureus models supports the hypothesis that [99m]Tc- ceftazidime is retained at infectious site because of its binding to bacterial cells [30].

**Table 2b.** Biodistribution of ^{99m}Tc-ceftazidime in body organs inflamed mice for live S.aureus at 15, 30, 60 and 120 min post injection

| Organ Time (min) | Live S.Aureus | | | |
|---|---|---|---|---|
| | **15** | **30** | **60** | **120** |
| **Blood** | 19.8±0.2 | 7.4±0.2 | 6.63±0.1 | 2.54±0.1 |
| **Inflammed Muscle** | **1.4±0.2** | **2.8±0.1** | **4.19±1.0** | **3.89±0.1** |
| **Normal Muscle** | **0.95±0.2** | **1.34±0.1** | **0.83±0.1** | **1.06±0.0** |
| **Bone** | 0.95±0.7 | 2.09±1.5 | 2.44±0.05 | 2.52±0.1 |
| **Stomach** | 1.02±0.1 | 3.3±0.2 | 1.18±0.1 | 3.15±0.1 |
| **Intestine** | 1.9±0.1 | 2.06±0.1 | 1.35±0.01 | 2.55±0.1 |
| **Kidneys** | 14.5±0.1 | 10.6±1.6 | 10.1±1.4 | 21.2±0.9 |
| **Liver** | 5.8±0.0 | 2.97±0.2 | 3.7±0.1 | 4.27±2.2 |
| **Spleen** | 1.3±0.2 | 1.77±0.1 | 1.03±0.0 | 1.81±0.2 |
| **Lung** | 4.7±0.7 | 1.35±0.5 | 1.95±01 | 2.6±0.1 |
| **Heart** | 5.37±0.2 | 1.09±0.2 | 1.09±0.2 | 2.37±0.1 |
| **Urine** | 3.2±1.4 | 62.8±5.5 | 26.1±3.2 | 18.1±5.1 |
| **T/NT** | **1.49±0.3** | **2.08±0.2** | **5.06±0.1** | **3.89±0.3** |

**Table 2c.** Biodistribution of ^{99m}Tc-ceftazidimebody organs inflamed mice for Killed S.aureus oil at 15, 30, 60 and 120 min post injection

| Organ Time (min) | Killed S.Aureus | | | |
|---|---|---|---|---|
| | **15** | **30** | **60** | **120** |
| **Blood** | 8.63±0.2 | 14.4±0.3 | 25.1±0.2 | 5.83±0.1 |
| **Inflammed Muscle** | **1.73±0.2** | **1.56±0.1** | **1.08±0.2** | **1.31±0.2** |
| **Normal Muscle** | **1.29±0.1** | **1.46±0.1** | **0.8±0.2** | **1.24±0.2** |
| **Bone** | 4.07±0.0 | 6.22±0.1 | 4.58±1.2 | 8.43±1.3 |
| **Stomach** | 6.15±0.0 | 0.82±0.0 | 0.6±0.0 | 2.57±0.0 |
| **Intestine** | 2.25±0.1 | 1.98±0.1 | 0.73±0.1 | 1.42±0.0 |
| **Kidneys** | 17.9±1.2 | 10.5±1.6 | 5.2±1.6 | 10.3±0.1 |
| **Liver** | 6.1±0.2 | 7.9±0.3 | 1.9±0.1 | 3.4±0.0 |
| **Spleen** | 2.37±0.1 | 6.6±0.1 | 1.3±0.0 | 1.4±0.1 |
| **Lung** | 2.9±0.2 | 1.9±0.1 | 0.9±0.1 | 2.34±0.1 |
| **Heart** | 3.26±0.1 | 4.9±0.2 | 1.04±0.2 | 1.9±0.1 |
| **Urine** | 2.97±5.1 | 3.3±6.1 | 30.8±5.4 | 39.8±1.1 |
| **T/NT** | **1.34±0.1** | **1.06±0.1** | **1.35±0.1** | **1.05±0.1** |

As a result, ^{99m}Tc-ceftazidime showed better yield and biodistribution for imaging of infection at early stages and distinguishing infection from sterile inflammation than the commercially available ^{99m}Tc-ciprofloxacin T/NT = 3.8 ± 0.4%.

# 3. Conclusions

[99m]Tc-ceftazidime was labeled easily using 1 mg Ceftazidime, 100 µl of $SnCl_2.2H_2O$ (10 µg Sn) as reducing agent, 3 mCiNa[99m]$TcO_4^-$, at pH 10 with a high labeling yield of 86.5 ±1.3%, using simple and instantaneous method with stability (up to 2 h) post labeling. ^{99m}Tc-ceftazidime was accumulated at the site of infection withT/NT ratio=5.03 higher than that of the commercially available ^{99m}Tc-ciprofloxacin (labeling yield ~ 80%, stable up to 2 h post labeling and T/NT=3.8). So ^{99m}Tc-ceftazidime showed better yield and biodistribution for imaging of infection at early stages and distinguishing infection from sterile inflammationthan ^{99m}Tc-ciprofloxacin.

# REFERENCES

[1] Gaurav Malviya, Erik F.J.de vries, Rudi A. Diercky and Alberto Signar, 2007: Radiopharmaceuticals for Imaging Chronic Lymphocytic Inflammation, vol 50:pp1:13.

[2] Simone Odilia Fernandes Diniz, Cristiano Ferrari Siqueira, David Lee Nelson, Josep Martin-Comin and Valbert Nascimento Cardoso, 2005: Technetium-99m Ceftizoxime kit preparation, Brazilian Archives of Biology and Technology, vol, 48: pp 89-96.

[3] David wareham, J. Michael and Stya Das (2005). Advances in Bacterial Specific Imaging. Brazilian archives of Biology and Technology; 48: 145-152.

[4] Zhang J, S, Guo H, Wang X (2010). Bioorg Med Chem Lett; 20:3781-3784.

[5] Lambrecht FY, Yilmaz O, Durkan K. Unak P, Bayrak E (2009). J Radioanal Nucl Chem 281:415-419.

[6] Fournier B, Zhao X, Lu T, Drlica k, Hooper DC (2000) Anti-microb Agents Chemother 44:2160.

[7] El-Ghany EA, El-Kolaly MT, Amine AM, El-Sayed AS, Abdel-Gelil F(2005) J Radioanal Nucl Chem 266:131-135.

[8] Motaleb MA (2007) J Radioanal Nucl Chem 272:167-171.

[9] Wareham D, Das S, Britton K(2000). Imaging Infection. In: Wilcox M. editor. Fast Facts-infection Highlights 1999-2000. Oxford: Health Press;81-87.

[10] DasSS, Hall Av, Wareham DW, Britton KE (2002). Infection Imaging with Radiopharmaceutical in the 21st Century. Braz Arch Biol Tech; 45, 25-37.

[11] Asikoglu M, Yurt F, Cagliyan O, Unak P, Ozkilic H (2000). Appl Radiat Iot 53:411-413.

[12] Singh, Baljinder; Sunil,Hejjaji V.; Sharma, Sarika; Prasad, Vikas; Kashyap, Raghav; Bhattacharya, Anisha; Mittal, Bhagwant R.; Taneja, Ashish; Rai, Rajeev; Goni, vijay G.; Aggarwal, Sameer; Gill, Shivinder S.; Bhatangar, Aseem; Singh, Ajay K (2001). Efficacy of indigenously developed single vial kit preparation of 99mTc-ciprofloxacin in the detection of bacterial infection; Eur J. Nucl Med; 28970: 822-30.

[13] Luigi. Zerilli, Luigi Cavenaghi, Alberto Bernareggi and Alessandro Assandri(1989), Teicoplanin Metabolism in rats, Antimicrobial Agents and Chemotherapy,P;1791-1794.

[14] Laken V, Boerman CJ, Oyenoc, Vande Ven WJG. Meer JWM, Corstens FHM, (2000). J. Nucl Med 41:463-469.

[15] Oyen WJG, Boerman OC, Corstens FHM (2001). J. Microbial Methods 47:151-157.

[16] Das SS, Hall Av, Wareham DW, Britton KE (2002). Infection Imaging with Radiopharmaceutical in the 21st Century. Braz Arch Biol Tech; 45, 25-37.

[17] Peter H, Nibbering, Mick M. Welling, Akke Paulusma-Annema, Carlo P. J. M. Brouwer, Antonella Lupetti and Ernest K. J. Pauwels (2004). [99mTc]-Labeled UBI 24-41 peptide for Monitoring the Efficacy of Antimicrobial Agents in Mice Infected with Staphylococcus aureus, J, Nucle. Med; 45:321-326.

[18] El-Ghany EA, Amin AM, El-Kawy OA, Amin. M (2007), J, Label Compound Radiopharm; 50: 23-31.

[19] Reitman S, Frankel SA (1957). A Colorimetric method for the determination of serum glutamic oxaloacetic and pyruvic transaminases Am. J. Clin. Pathol., 28(1):56-63.

[20] Rhods BA, Wagner HN (1974), J. Nucl. Med; 15:213-214.

[21] Roohi S, Mushtaq A, Jehangir M, Ashfaq MS (2006), J Radioanal Nucl Chem; 267: 561-566.

[22] Shah SQ, Khan AU, Khan MR (2010) Appl Radiat Isot; 68: 2255-2260.

[23] Lambrecht FY, Yurt F, Durkan K, Unak P (2008), J Radioanal Nucl Chem; 275: 161-164.

[24] Srivastava Sc, Richard P (1983), Technetium Label Compounds In: Rayudu GVD(ed) Radiotracers for Medical Application CRC series in Radiotracer in Biology and Medicin, CRC press, Boca Ratom, PP:107-185.

[25] Wardell JL (1994), Tin in organic chemistry, In: King RB(ed) Encyclopedia of inorganic chemistry, vol 8. Wiley, New York, pp:4159-4197.

[26] Sebnem Atici, Ismail Cinel, Leyla Cinel, Nurcan Doruk, Gulcin Eskandari and Ugur Oral (2005), Liver and Kidney Toxicity in chronic use of opioids: An experimental long term treatment model, J, Bio Sci;30(2), p, 245-252.

[27] Dodds, M. W., and Law, S. K. A(1998), the phlogeny and evolution of the thioester bond containing protein $C_3$, $C_4$ and $\alpha_2$ -macroglobulin, Immunal. Rev., 166(1):15-26.

[28] Taina Mosca, Maria C. S. de Menezes, Particia C. L. Dionigi, Roberto Stirbulov, Wilma C. N. forte(2012). $C_3$ and $C_4$ Complement system components as biomarkers in the intermittent atopic asthma diagnosis, J. de pediatria, vol, 87, No,6,p;512-515.

[29] Jing Zhang, Jingyun Koh, Jinhua Lu, Steffen Thiel, Benjamin S.H. Leong, Sunil Sethi, Cynthia Y.X. He, Bow Ho, Jeak L. Ding (2009): Local Inflammation Induces Complement Crosstalk Which Amplifies the Antimicrobial Response. Complement Cross Resolves Inflammation, PLOS Pathogens Journal, vol, 5, Issue 1, p;1-14.

[30] Peter Laverman, Chantal P, Bleeker Rovers, Frans H. M. Corstens, Otto C. Boerman and Wim J. G. Oyen (2008). Development of infection and inflammation targeting compounds, current radiopharmaceuticals; 1: 42-48.