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Evaluation of ^{99m}Tc-amoxicillin sodium as an infection imaging agent in bacterially infected and sterile inflamed rats

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Abstract Bacterial infection is one of the major causes of morbidity and mortality especially in developing countries. The aim of this study was to develop a new radiopharmaceutical for imaging infection. The labeling conditions were optimized, and lyophilized kits were developed for instant preparing. The stability of ^{99m}Tc-AMOX in human serum was identified, sterility and pyrogenicity of the radiopharmaceutical were estimated, gamma scintigraphy and in vivo biodistribution with infected rats were investigated. The promising properties of ^{99m}Tc-AMOX combined with the development of reliable and instant lyophilized kit afford the opportunity of inflammatory process imaging.

Keywords ^{99m}Tc · Amoxicillin sodium · Radiolabeling · Radiolabeled antibiotic · Infection imaging · Inflammation imaging

Introduction

Globally, acute, subacute and chronic bacterial infections are major causes of mortality and morbidity [1]. The in-time diagnosis of bacterial infection is extremely significant for the accurate treatment [2]. Clinicians use variety of physical examinations, laboratory and radiological tests to aid the diagnosis and make decisions [1]. Although the radiological imaging techniques including computed tomography (CT), nuclear magnetic resonance (NMR) and ultrasonography (US) are commonly used, these techniques are limited use due to insignificant anatomical changes in early stage of the disease. In patients with serious underlying conditions, the identification of infection at early stage of the disease is critical for a favorable outcome [1–4].

Infection detection by nuclear medicine imaging techniques based on pathophysiological and pathobiological changes which appear much earlier than anatomical changes in the infection process [5, 6]. Nuclear medicine techniques require reliable radiopharmaceuticals that can selectively concentrate in the site of infection [7]. ^{67}Ga-citrate and ^{111}In-labeled leucocytes have shown promising diagnostics results but awkward methods of formulation and high radiation dose limit their use in clinics [2]. Since the favorable properties such as 140 keV pure gamma energy and the half-life of 6 h, ^{99m}Tc is the most widely used radionuclide for diagnostic applications in nuclear medicine departments. The use of ^{99m}Tc labeled antibiotics for infection imaging is promising because of their specific activity against bacteria. In recent years ^{99m}Tc labeled ciprofloxacin (infecton) were evaluated as an infection imaging agent. [8, 9]. Two vial cold kits of infecton which was further modified to a single vial, has some bottlenecks in the use of the kit such as need for filtration during reconstitution caused additional radiation burden and cause the loss of radioactivity [5, 9–11].

To develop a better infection imaging which will accumulate efficiently to inflammatory foci, clear rapidly from background tissue, discriminate between bacterial infection and sterile inflammation, cost low and prepare

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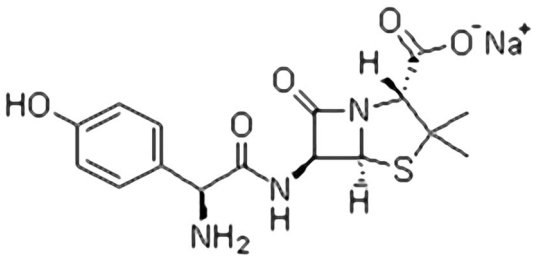

Fig. 1 Chemical structure of AMOX

easily we labeled amoxicillin sodium (AMOX) with ^{99m}Tc. AMOX is a semisynthetic antibiotic and is a member of the penicillinase-stable group of penicillins derived from the penicillin nucleus (Fig. 1) [12].

In this study, we prepare ^{99m}Tc-AMOX in a simple radiochemical method with good labeling efficiency and evaluate the ready to use cold kit formulation thus making it available to the other nuclear medicine centers. The stability of ^{99m}Tc-AMOX in human serum was identified, sterility and pyrogenicity of the radiopharmaceutic were estimated and gamma scintigraphy studies with bacterial infected and sterile inflamed rats were investigated.

Materials and methods

Materials

All chemicals and solvents were used without further purification. AMOX was a gift from AppliChem, (Germany). Stannous tartrate and stannous chloride dehydrate were purchased from Sigma-Aldrich (USA) and ascorbic acid was purchased from Sigma-Aldrich (United Kingdom). ^{99m}Tc was eluted from the ^{99}Mo (^{99}Mo)/^{99m}Tc-generator (Nuclear Medicine Department of Ege University). Thioglycollate Broth (TB) and Tryptic Soy Broth (TSB) mediums and all solvents were obtained from Merck (Germany). Dose Calibrator (Atomlab 100, Biodex Medical Systems) was used for counting radioactive samples. *Escherichia coli* (ATCC 25922) bacteria were obtained from Pharmaceutical Microbiology Department of Ege University. The Animal Ethics Committee of the Ege University gave approval for the animal experiments (Number: 2010-37, 2010). Results are reported as mean ± standard error.

Radiolabeling studies

To investigate the optimum radiolabeling conditions, radiolabeling was tested with different concentrations of reducing (stannous chloride and stannous tartrate) and antioxidant (ascorbic acid) agent. Radiochemical analysis was performed with Radio Thin Layer Chromatography (RTLC) and Radio High Performance Liquid Chromatography (RHPLC) studies. Two different freeze dry kits were formulated with optimum labeling conditions and stability of the kits were performed.

Effect of reducing agent on labeling

Two different concentrations of AMOX solution were prepared in saline. To lower concentrated stock solution (5 mg/1 mL), stannous chloride was added under an atmosphere of bubbling nitrogen. Reduction of ^{99m}Tc was performed with different amount of stannous chloride in 0.1 N HCl (30, 80, 100, 150 and 200 μg). To higher concentrated stock solution (10 mg/1 mL), different amount of stannous chloride in 0.1 N HCl (100, 150 and 200 μg) was added under an atmosphere of bubbling nitrogen. Radiolabeling was performed with ^{99m}Tc (37 MBq) in saline (0.1 mL) and solution was allowed to stand at room temperature for 15 min prior to radiochemical analyses.

Reducing agent effect on labeling efficiency was also performed with the formulations described above by using stannous tartrate instead of stannous chloride.

Effect of antioxidant agent on labeling

To examine the effect of antioxidant agent on labeling efficiency and stability of the complex, labeling studies were performed in the absence and presence of antioxidant agent. AMOX solution were prepared (10 mg/1 mL), in three groups of vials. 100, 150 and 200 μg stannous chloride was added to each individual group. Each group has three vials and labeling was performed in the absence and presence (1.5 and 3.0 mg) of ascorbic acid. Freshly eluted 37 MBq ^{99m}Tc was added to each vial. The vials were shaken for 30 s, filtered through a 0.22 μm pore size filter and incubated for 15 min at room temperature. The labeling efficiency was analyzed by RTLC.

Antioxidant agent effect on labeling efficiency was also performed with ideal formulation by using stannous tartrate instead of stannous chloride.

Effect of incubation time on labeling

After radiolabeling the radiochemical purity of the complexes were investigated with RTLC studies which performed at different time intervals (5, 15, 30, 45 and 60 min postlabeling).

Effect of pH on labeling

The radiochemical purity of a ^{99m}Tc radiopharmaceutical is highly dependent on the pH of the kit mixture. To investigate the effect of the pH on labeling efficiency, ^{99m}Tc-AMOX solution pH was adjusted to 4.8–7.4 by adding 0.1 N NaOH.

Stability of ^{99m}Tc-AMOX in saline

100 μL of ^{99m}Tc-AMOX reaction medium was added to 1 mL of saline. The mixture was incubated at 37 °C for 24 h. Two drops of sample was spotted on chromatographic strips at different time intervals up to 24 h and RTLC studies were performed to determine the stability of ^{99m}Tc-AMOX in saline.

Stability of ^{99m}Tc-AMOX in Human Serum

100 μL of ^{99m}Tc-AMOX reaction medium was added to 1 mL of freshly prepared human serum. The mixture was incubated at 37 °C for 24 h. Two drops of sample was spotted on chromatographic strips at different time intervals up to 24 h and RTLC studies were performed to determine the stability of ^{99m}Tc-AMOX in human serum

RTLC procedure

Silica gel coated fiber sheets (SG) and instant thin layer silica gel papers (ITLC-SG) were used as stationary phase. Free and Reduced/Hydrolyzed (R/H) ^{99m}Tc were determined by using methyl ethyl ketone (MEK) and butanone/ethanol/water (B/E/W) (35/35/30) as the mobile phases respectively. The radioactivity on plates was measured using a TLC scanner (Bioscan AR 2000), and % radiochemical purity (RP) of ^{99m}Tc-AMOX was calculated from the following equation by subtracting from 100 the sum of measured impurities percentages.

$$\mathrm{RP}\ (\%) = 100 - \left(\mathrm{Free}^{99m}\mathrm{Tc}\ (\%) + \mathrm{R/H}^{99m}\mathrm{Tc}\ (\%)\right)$$

RHPLC analysis

The compounds were further analyzed by an Ultra-HPLC system equipped with a C_{18} column connected to a photodiode array detector (PDA) and additional NaI gamma detector for the ^{99m}Tc compounds. The flow rate was 1.0 mL/min for analytical runs. In all runs the eluent was 0.1 % TFA in H_2O (solvent A) and 0.1 % TFA in ACN (solvent B). For the analytical control and semi preparative separation the method was as follows: 0–2.5 min 95 % solvent A-5 % solvent B; 2.5–5 min 50 % solvent A 50 %-solvent B; 5–7.5 min 20 % solvent A 80 %-solvent B; 7.5–10 min 5 % solvent A-95 % solvent B.

Freeze dry kit formulation and radiolabeling

After observing the effect of different parameters on labeling, subsequently two lots were prepared as follows: Lot A was prepared by mixing 10 mg AMOX, 200 μg stannous tartrate and 3 mg ascorbic acid. Lot B was prepared by mixing 10 mg AMOX, 200 μg stannous chloride and 3 mg ascorbic acid. Both kits solution were filtered through a 0.22 μm pore size filter to glass vials, filtered solutions were frozen in a freezer at −80 °C and lyophilized at −20 °C for 24 h.

In vitro radiolabeling studies were performed with 37 MBq ^{99m}Tc for the regard to radiation safety of personnel and the environment. Freeze dry were labeled with 37–370 MBq ^{99m}Tc.

Stability of the freeze dry kits

The kits were stored at +5 ± 3 °C (in a refrigerator) and +25 °C ± 2 °C/60 %RH (relative humidity) ± 5 % (in a stability cabin). The labeling efficiency of ^{99m}Tc-AMOX in each kit was checked at different time intervals up to 6 months. The kits were reconstituted with 37 MBq of ^{99m}Tc-pertechnetate and the radiochemical purity was determined by RTLC and RHPLC studies.

Microbiological analysis of ^{99m}Tc-AMOX Kit

Sterility test

The sterility of the kits was tested by direct inoculation method according to the European Pharmacopeae 6.0 recommendations. The dilutions of the kits were aseptically inoculated to the sterilized TB and TSB medium vials, and incubated at 35 ± 2 °C. At the end of the incubation, absence of clearly visible growth of microorganisms in the vials exhibited the sterility.

Pyrogenicity test

Pyrogenicity test was performed by the gel clot method which based upon the reaction between bacterial endotoxin and the specific lysate. In the presence of endotoxin the gel is formed via a clotting reaction and the sample is fail. Endotoxin limit value of the kit and Maximum Valid Dilution (MVD) was estimated according to European Pharmacopeia 6.0. The LAL test is performed by Pyrotell (Associates of Cape Cod, Incorporated, Falmouth, Massachusetts) Gel-Clot formulation.

Animal studies

Wistar albino rats (200–250 g) were used for animal studies. Experiments with rats were performed according to a protocol approved by Animal Ethics Committee of the Ege University.

Induction of bacterial infection model

E.coli suspension was used to create infection model. 200 μL (4×10^{10}cfu/1 mL) *E.coli* suspension was intramuscularly injected into the right thigh muscle of rats ($n = 6$). Then the infection was allowed to develop for 24 h.

Induction of sterile inflammation model

200 μL Turpentine oil was intramuscularly injected into the left thigh muscle of rats ($n = 6$). Then the inflammation was allowed to develop for 24 h.

Scintigraphic imaging studies of ^{99m}Tc-AMOX

After the infection and sterile inflammation focus were allowed to develop for 24 h swelling appeared and gamma scintigraphy studies were performed. ^{99m}Tc-AMOX (3.7 MBq) was intravenously injected via tail vein to the rats. During the scintigraphy studies animals were under anesthetize with Ketamine/Xylazin cocktail. The scintigraphic images were obtained with a dual head gamma camera (Infinia General Electric) equipped with a low-energy high-resolution collimator viewing the whole body of rats. After administration of radiopharmaceuticals, serial static images were acquired in a 256 × 256 matrix for 60 s each, at different time intervals (5 min, 1, 2, 3, 4 and 5 h post injection).

Biodistribution studies

5 h after injection, the rats were sacrificed and biodistribution was determined. Samples of infected muscle, inflamed muscle, healthy muscle, blood, liver, spleen, lung, kidney, stomach, intestine, urine and heart were weighed and the radioactivity was measured using a gamma counter (Sesa Uniscaller). The results were expressed as the percentage of injected dose per gram of tissue (%ID/g).

Statistical analysis

The calculation of means and standard deviations were made on Microsoft Excel. Oneway Anova was used to determine statistical significance. Differences at the 95 % confidence level ($p < 0.05$) were considered significant.

Results and discussion

Radiolabeling studies

A new, simple, rapid and efficient direct method for labeling of AMOX with ^{99m}Tc was developed. Labeling efficiency of the ^{99m}Tc-AMOX was assessed by both RTLC and RHPLC studies. Two solvent systems were used to distinguish and quantify the amounts of radioactive contaminants.

Table 1 Rf values of ^{99m}Tc-AMOX in mobile phases

	SG MEK	ITLC-SG B/E/W
Free ^{99m}Tc	0.8–1.0	0.8–1.0
R/H ^{99m}Tc	0.0	0.0
^{99m}Tc –AMOX	0.0	0.8–1.0

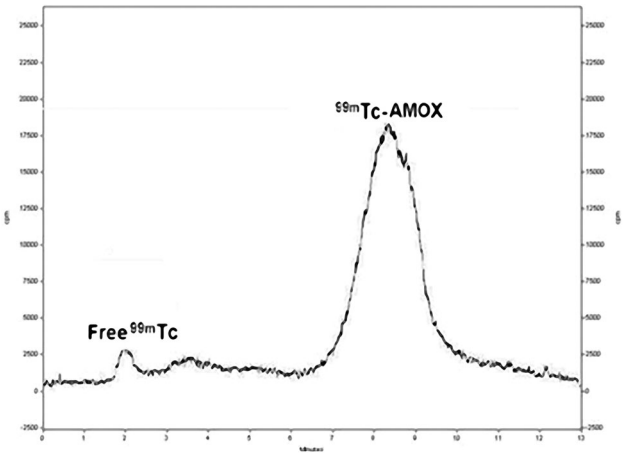

Fig. 2 The RHPLC chromatogram of 99mTc-AMOX

In RTLC, using MEK as the solvent, free ^{99m}Tc moved with the solvent front, while ^{99m}Tc-AMOX and R/H ^{99m}Tc remained at the spotting point. R/H ^{99m}Tc was determined by using B/E/W (35/35/30) as the mobile phase where the R/H ^{99m}Tc remained at the point of spotting while free ^{99m}Tc and ^{99m}Tc-AMOX moved with the solvent front. The Rf values of ^{99m}Tc-AMOX in mobile phases was presented in Table 1.

The radiochemical purity of ^{99m}Tc-AMOX was found >95 %. The RHPLC chromatogram was presented in Fig. 2 and showed two peaks, first one was corresponds to free ^{99m}Tc, while the second peak for ^{99m}Tc-AMOX.

^{99m}Tc may be formed by interactions between electron donor atoms such as nitrogen, oxygen, sulfur and reduced technetium. Due to presence of electron donor atoms in amoxicillin structure the reduced sodium pertechnetate can easily react with this ligand and a complex is formed. To determine exact structure of the complex further research is necessary.

Effect of reducing agent on labeling

To investigate the effect of reducing agent type on labeling yield, labeling experiments were performed with the same

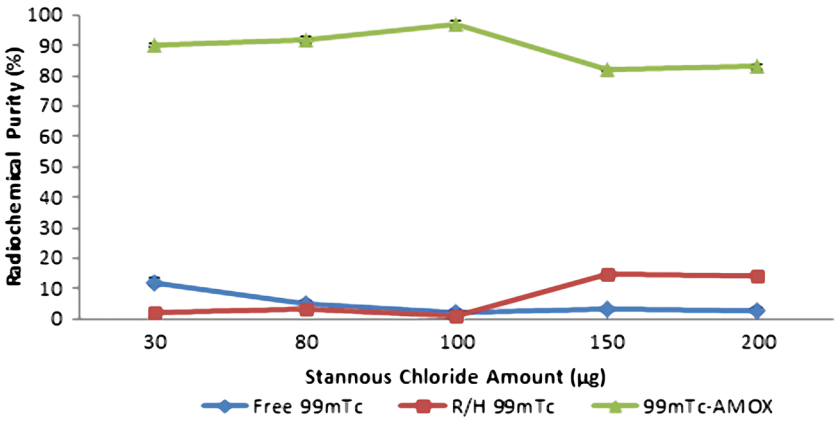

Fig. 3 The effect of reducing agent concentration on the radiochemical purity of 99mTc-AMOX (5mg/1mL)

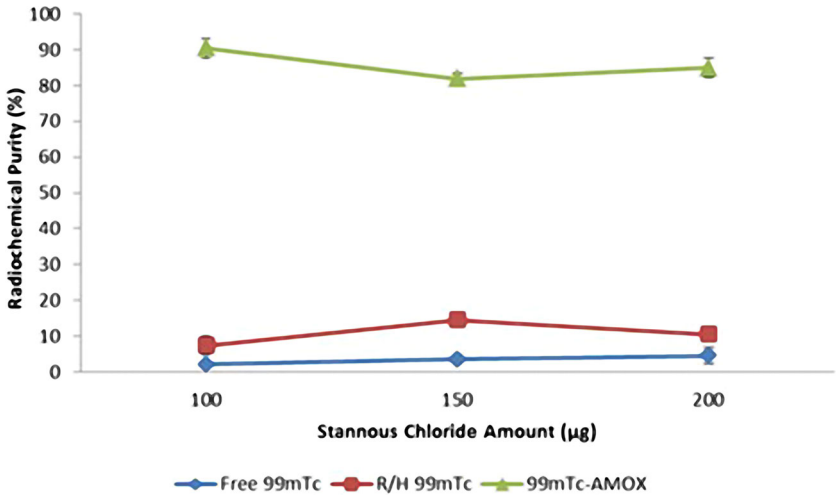

Fig. 4 The effect of reducing agent concentration on the radiochemical purity of 99mTc-AMOX (10mg/1mL)

amounts of active ingredient, reducing agent and radionuclide including formulations. Two groups of formulation were prepared. While first group include lower concentration of AMOX (5 mg/1 mL), second group include higher concentration of (10 mg/1 mL) instead. Comparative results for both formulations were shown in Figs. 3 and 4 respectively.

The effect of reducing agent concentration on the radiochemical purity was evaluated and optimum reducing agent amount found 200 µg. Under these conditions labeling efficiency was around 90 %.

According to the results there were no differences found between the labeling efficiency of same amounts of stannous tartrate or chloride included formulation. So two lots of freeze dry kit were prepared and reducing agent type on the stability o kits was studied.

Effect of antioxidant agent on labeling

In the presence of ascorbic acid, stability of the complex was slightly increase while labeling efficiency for early hours was not affected significantly. R/H ^{99m}Tc percentage was decreased in the presence of ascorbic acid. The results in these experiments revealed that, maximum radiochemical purity was obtained with 10 mg AMOX, 200 µg reducing agent (stannous chloride, stannous tartrate) and 3 mg ascorbic acid including formulations (Tables 2, 3, 4 and 5). Effect of incubation time, pH and stability of these formulations were investigated to evaluate the optimum labeling conditions for AMOX.

Effect of incubation time on labeling

200 µg stannous chloride or stannous tartrate and 3 mg ascorbic acid including formulations were labeled with 37 MBq ^{99m}Tc. The radiochemical purity of the complexes was investigated with RTLC studies at 5, 15, 30, 45 and 60 min postlabeling (Table 6). According to the experiments, the formation of the ^{99m}Tc-AMOX complex was very fast and reached the radiochemical purity over 95 % in 15 min after radiolabeling.

Table 2 Radiochemical purity of 100 μg stannous chloride including lower concentrated (5 mg/1 ml) formulations in the absence and presence of ascorbic acid

Time (h)	Radiochemical purity (%)		
	Absence of ascorbic acid	Ascorbic acid (mg)	
		1.5	3.0
0.25	90.48 ± 2.77	90.18 ± 0.56	90.48 ± 0.42
1	79.56 ± 1.16	91.33 ± 1.21	89.92 ± 0.51
2	89.47 ± 2.16	89.66 ± 1.42	90.00 ± 0.16
3	79.79 ± 3.33	88.53 ± 1.35	88.53 ± 0.25
4	70.04 ± 1.23	88.50 ± 0.59	88.57 ± 0.49
5	69.52 ± 0.56	86.89 ± 1.24	87.91 ± 1.25
6	76.31 ± 0.59	84.99 ± 1.16	85.99 ± 1.38

Table 3 Radiochemical purity of 150 μg stannous chloride including lower concentrated (5 mg/1 ml) formulations in the absence and presence of ascorbic acid

Time (h)	Radiochemical purity (%)		
	Absence of ascorbic acid	Ascorbic acid (mg)	
		1.5	3.0
0.25	81.90 ± 1.42	87.12 ± 1.24	89.26 ± 1.54
1	82.90 ± 1.31	86.97 ± 1.67	88.41 ± 1.67
2	77.67 ± 0.45	85.70 ± 0.73	90.60 ± 0.72
3	72.70 ± 0.87	87.55 ± 1.29	89.36 ± 1.22
4	70.07 ± 1.54	88.42 ± 1.52	88.69 ± 1.34
5	65.51 ± 1.14	87.09 ± 1.82	91.77 ± 0.64
6	66.06 ± 0.81	85.93 ± 0.56	88.66 ± 0.59

Table 4 Radiochemical purity of 200 μg stannous chloride including lower concentrated (5 mg/1 ml) formulations in the absence and presence of ascorbic acid

Time (h)	Radiochemical purity (%)		
	Absence of ascorbic acid	Ascorbic acid (mg)	
		1.5	3.0
0.25	84.91 ± 2.68	94.95 ± 0.54	97.12 ± 1.06
1	82.23 ± 2.31	94.92 ± 0.19	96.60 ± 0.33
2	76.39 ± 0.97	94.58 ± 1.64	97.59 ± 1.42
3	75.04 ± 2.25	94.19 ± 1.28	97.59 ± 1.18
4	76.81 ± 0.98	93.01 ± 1.33	96.08 ± 1.07
5	70.57 ± 1.79	93.02 ± 0.29	96.27 ± 1.26
6	73.15 ± 2.82	92.95 ± 1.50	96.43 ± 1.38

Effect of pH on labeling

The effect of pH on radiochemical purity was examined at pH 4.8-7.4. According to the experiments results the pH of the reaction medium was not found to play an important role in the labeling process (Fig. 5). While keeping other reaction conditions constant and varied the pH of the reaction from 4.8 to 7.4 radiochemical purity was slightly decreased.

Table 5 Radiochemical purity of 200 μg stannous tartrate including lower concentrated (5 mg/1 ml) formulations in the absence and presence of ascorbic acid

Time (h)	Radiochemical purity (%)		
	Absence of ascorbic acid	Ascorbic acid (mg)	
		1.5	3.0
0.25	89.54 ± 0.12	92.04 ± 0.25	96.57 ± 0.51
1	85.25 ± 0.25	92.14 ± 0.15	96.04 ± 1.80
2	82.43 ± 0.64	91.87 ± 1.54	97.24 ± 1.22
3	79.25 ± 0.82	92.61 ± 0.46	96.27 ± 1.38
4	77.59 ± 0.42	91.43 ± 0.91	96.23 ± 1.26
5	75.81 ± 1.55	91.95 ± 1.05	96.76 ± 0.59
6	72.46 ± 1.76	91.14 ± 0.89	96.17 ± 0.48

Table 6 Effect of incubation time on radiochemical purity of ^{99m}Tc-AMOX

Time (min)	Reducing agent	
	Stannous chloride Radiochemical purity (%)	Stannous tartrate
5	92.18 ± 0.52	91.18 ± 0.18
15	97.12 ± 1.06	96.57 ± 0.51
30	97.14 ± 0.36	96.95 ± 0.15
45	96.89 ± 0.25	97.01 ± 1.14
60	96.60 ± 0.33	96.04 ± 1.80

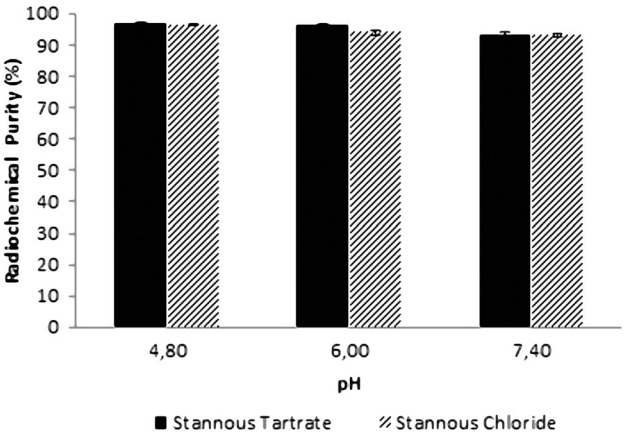

Fig. 5 Effect of the pH on radiochemical purity of 99m Tc-AMOX

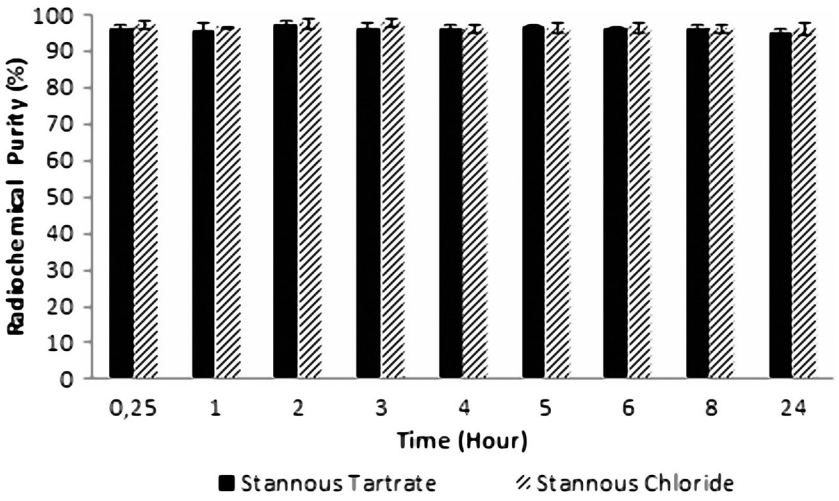

Fig. 6 The stability of 99mTc-AMOX in saline up to 24 hours

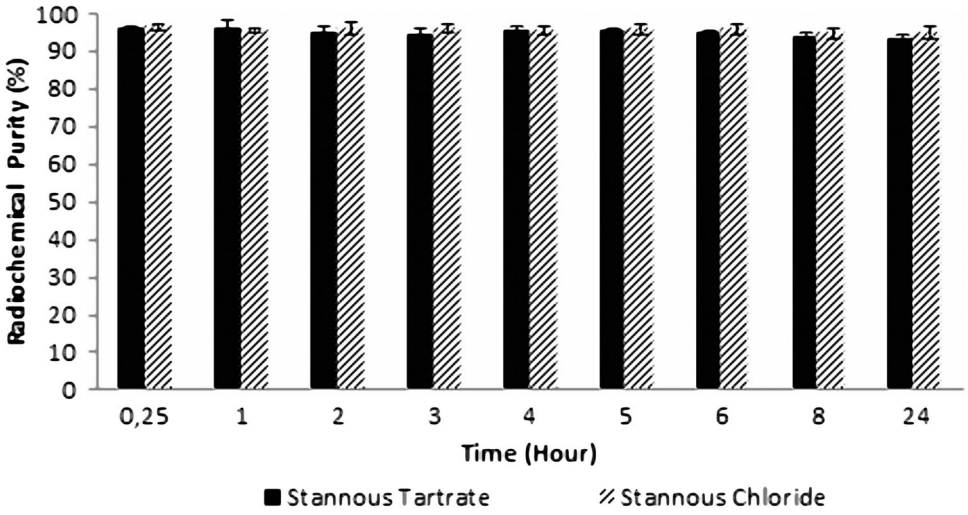

Fig. 7 The stability of 99mTc-AMOX in human serum up to 24 hours

Stability of ^{99m}Tc-AMOX in saline

^{99m}Tc-AMOX was found stable during to 24 h incubation in saline (Fig. 6).

Stability of ^{99m}Tc-AMOX in Human Serum

^{99m}Tc-AMOX was found stable during to 24 h incubation in human serum (Fig. 7).

Stability of the freeze dry kits

Kits were labeled with 37, 185 and 370 MBq ^{99m}Tc. Slightly decrease in radiochemical purity was observed with increasing of radioactivity (Fig. 8).

The stability of the freeze dry kits which stored in +5 ± 3 °C and +25 ± 2 °C/60 %RH ± 5 % was determined at different time intervals up to 6 months. According to experiments, both kits (Lot A and Lot B) were found stable up to 6 months without any significant decrease in radiochemical purity (Fig. 9).

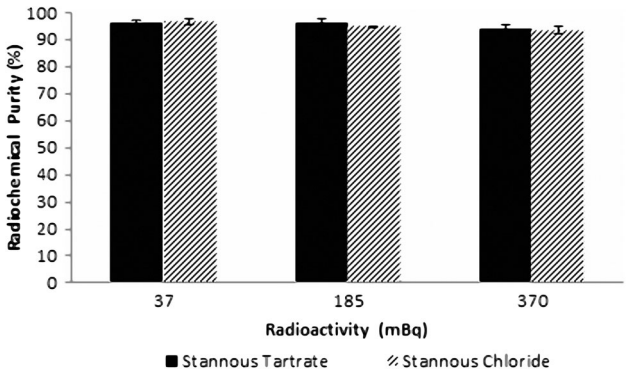

Fig. 8 Effect of radiation dose on radiochemical purity of 99mTc-AMOX

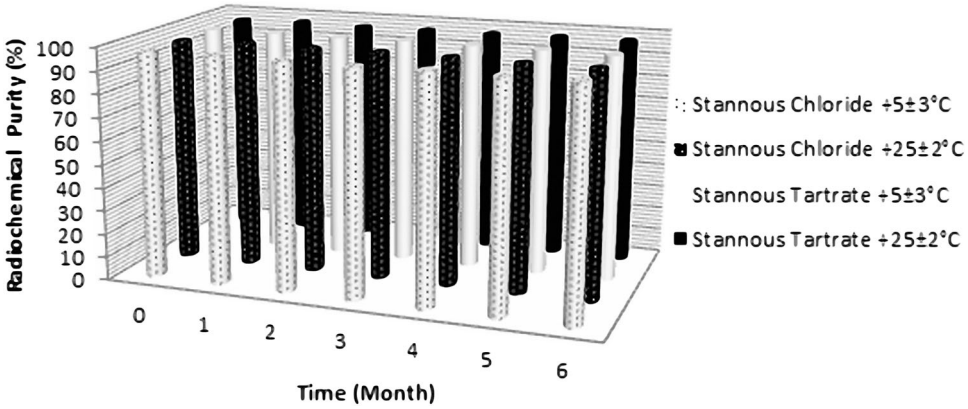

Fig. 9 Radiochemical purity of freeze dried kits at different incubation conditions and periods

The freeze dry kits developed in this study were found to be stable with a shelf–life of 6 months when preserved at both at $+5 \pm 3$ °C and $+25 \pm 2$ °C/60 %RH $\pm$ 5 %. Based on the findings of this study, by the reconstitution of these kits the complex easily formed without any requirement for boiling and post-labeling purification. Unfortunately there were no statistical differences found for labeling efficiency and stability of the different reducing agent included kits, 200 μg stannous tartrate included formulation was found optimal since to have higher labeling yield.

Microbiological analysis of ^{99m}Tc-AMOX Kit

According to sterility test, since there was no growth in batches, kits were sterile. Also gel clot test showed that kits were pyrogen free.

Scintigraphic imaging studies of ^{99m}Tc-AMOX

The uptake of ^{99m}Tc-AMOX following intravenous administrations was assessed on static images. The images depicted rapid distribution throughout the body and uptake in the bacterial infected and sterile inflamed thigh muscle within 1 hour after injection. As can be seen in Fig. 10 there was a higher activity in both bacterial infected and sterile inflamed thigh muscle as compared to healthy thigh muscle.

For quantitative evaluation, regions of interest were drawn around the target (infected and inflamed thigh muscle) and non-target (healthy thigh muscle) regions of the rats. The ^{99m}Tc-AMOX uptake was calculated by dividing the average counts per pixel within the region of target to the average counts per pixel within the region of non-target (Table 7). These ratios indicated a significant uptake of ^{99m}Tc-AMOX in infection and inflammation foci. The data suggest that ^{99m}Tc-AMOX remained at the infection and inflammation foci during the whole experiments, there being no statistically significant differences in the ratios during the studied period ($p \geq 0.05$).

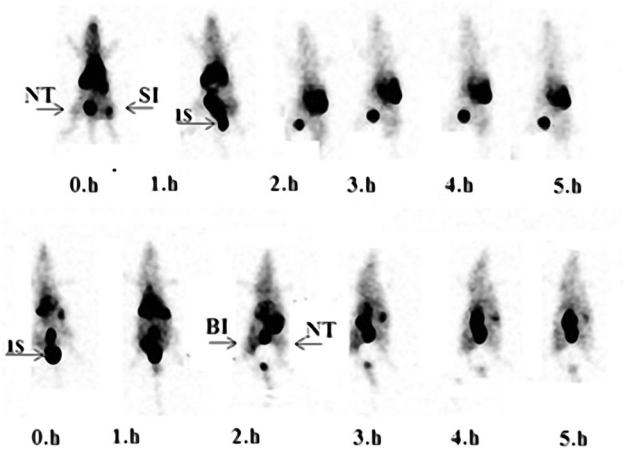

Fig. 10 The scintigrams of 99mTc-AMOX injected bacterial infected and sterile inflamed rats at different time intervals (SI=Sterile Inflamed Foci, BI= Bacterial Infected Foci NT=Non-Target Extremity, IS=Injection Site)

Table 7 Target/non-target ratios from ROI Analysis ^{99m}Tc-AMOX at different time intervals

Time after injection (h)	Infected thigh muscle (target/non-target)	Inflamed thigh muscle (target/non-target)
0	2.35 ± 0.59	1.94 ± 0.09
1	4.64 ± 1.51	2.94 ± 1.00
2	2.46 ± 0.19	2.61 ± 0.70
3	2.33 ± 0.99	2.41 ± 0.12
4	3.19 ± 1.26	2.73 ± 1.74
5	4.79 ± 2.82	3.51 ± 1.21

Biodistribution studies

Based on data presented in Tables 8 and 9, biodistribution of ^{99m}Tc-AMOX was determined for lung, kidney, spleen, intestine, heart, blood, liver stomach infected, inflamed and healthy thigh muscle. Moderate clearance from the kidney 1.34 ± 0.96–0.88 ± 0.13 %ID/g was observed. The

Table 8 Biodistribution of ^{99m}Tc-AMOX in bacterial infected rats 5 h post injection (%ID/g)

Organ	%ID/g	Organ	%ID/g
Lung	0.08 ± 0.05	Heart	0.07 ± 0.05
Kidney	1.34 ± 0.96	Blood	0.10 ± 0.07
Spleen	0.05 ± 0.02	Liver	0.09 ± 0.06
Small intestine	0.07 ± 0.05	Stomach	0.10 ± 0.06
Large intestine	0.13 ± 0.10	Infected muscle	0.15 ± 0.13
		Healthy muscle	0.09 ± 0.06

Table 9 Biodistribution of ^{99m}Tc-AMOX in sterile inflamed rats 5 h post injection (%ID/g)

Organ	%ID/g	Organ	%ID/g
Lung	0.05 ± 0.01	Heart	0.05 ± 0.01
Kidney	0.88 ± 0.13	Blood	0.09 ± 0.03
Spleen	0.04 ± 0.01	Liver	0.07 ± 0.01
Small intestine	0.49 ± 0.62	Stomach	0.30 ± 0.14
Large intestine	0.03 ± 0.01	Inflamed muscle	0.07 ± 0.02
		Healthy muscle	0.04 ± 0.01

presence of high activity in the kidney suggests the urinary system being the major route of excretion of the administered radiopharmaceutical. Amoxicillin has a mean elimination half-life of approximately 1 h and 60–70 % of the amoxicillin and is excreted unchanged in urine during the first 6 h after administration of a single 500/100 bolus intravenous injection. Various studies have found the urinary excretion to be 50-85 % for amoxicillin over a 24 h period [15]. So the authors can discuss the de-corporate ^{99m}Tc may be responsible for the gastrointestinal radioactivity 5 h after administration. The ^{99m}Tc-AMOX uptake was found 0.15 ± 0.13 %ID/g (Table 9), 0.07 ± 0.02 %ID/g for infected and inflamed thigh muscle of rats respectively.

Conclusion

AMOX, is s a member of the penicillinase-stable group of penicillins derived from the penicillin nucleus. So far various antibiotics were labeled with ^{99m}Tc. AMOX was previously labeled by Örümlü [13, 14]. The aim of this study was to standardize and develop a new, simple and ready to use kit of AMOX for radiolabeling with ^{99m}Tc. ^{99m}Tc-AMOX was developed and standardized under varying conditions of reducing and antioxidant agent concentration, pH, radioactivity dose and reducing agent type. Labeling studies were performed by changing the selected parameters one by one and optimum labeling conditions were determined. After observing the conditions for maximum labeling efficiency and stability, lyophilized freeze dry kits were prepared accordingly.

Simple method for radiolabeling of AMOX with ^{99m}Tc has been developed and standardized. Labeling efficiency of ^{99m}Tc-AMOX was assessed by both RTLC and RHPLC and found higher than 90 %. The resulting complex was quite stable and labeling of >90 % was maintained for up to 6 h. Two different freeze dry kits was developed and evaluated. Based on the data obtained from this study, both products was stable for 6 months with high labeling efficiency. To examine the role of ^{99m}Tc-AMOX in imaging of infection at early stage, in vivo studies are in progress.

The labeled compound was found to be stable in human serum up to 24 h. The prepared cold kit was found sterile and pyrogen free. The bacterial infection and sterile inflammation imaging capacity of ^{99m}Tc-AMOX was evaluated. Based on the in vivo studies, ^{99m}Tc-AMOX has higher uptake in infected and inflamed thigh muscle than healthy muscle. The data suggest that ^{99m}Tc-AMOX remained at the infection and inflammation foci during the whole experiments, there being no statistically significant differences in the ratios during the studied period ($p \geq 0.05$).

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