Preparation, biodistribution and scintigraphic evaluation of ^{99m}Tc-lincomycin

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Abstract: A complex of lincomycin was synthesized with technetium-99m. The synthesis was carried out by using $SnCl_2.2H_2O$ as reducing agent and ascorbic acid as stabilizer. The effect of various parameters such as amount of ligand/reducing agent, pH value and reaction time on radio labeling process was studied. The characterization of the ^{99m}Tc-Lincomycin was performed by HPLC and electrophoresis Biodistribution studies were carried out by analyzing the model of bacterial infectious rats (Sprague-Dawley). The uptake of infectious lesions at different time interval was also studied by using scintigraphic technique. The complex showed effective target to non-target ratio for various inflammatory or infectious lesions. The ^{99m}Tc-Lincomycin effective binding to living bacteria and could be used successfully as an infection imaging agent.

Keywords: ^{99m}Tc-Lincomycin, biodistribution, ligand/reductant ratio, *E. coli,* infectious lesions.

INTRODUCTION

Techentium-99m labeled antibiotics has been proposed as a radio pharmaceutical that could distinguish among bacterial infection, non-bacterial infection and sterile inflammation. (Essouissi *et al*., 2010; Gallagher *et al*., 2006; Meléndez-Alafort *et al*., 2004). Being able to distinguish between bacterial infection and other inflammations with a simple scintigraphic and biodistribution could have a major impact on the clinical management of patients with suspected bacterial infection. Many radio pharmaceuticals have been reported to detect and treat various infections. These include ^{99m}Tc-dextran (Bhatnagar *et al*., 1995), ^{99m}Tc-glucoheptonate (Passamonte *et al*., 1983), ^{99m}Tc-human immunoglobulin (Buscombe *et al*., 1990), ^{99m}Tc-nanocolloid (Roohi *et al*., 2006b), ^{99m}Tc-MIBI (Onsel *et al*., 1996), ^{99m}Tc-tetrofosmin (Degirmenci *et al*., 1998), ^{99m}Tc-Doxorubicin (Faheem *et al*., 2012), ^{99m}Tc-perrehnate(Bokhari *et al*., 2012), ^{99m}Tc-Daunorubicin (Faheem *et al*., 2013),^{99m}Tc-Ciprofloxacin(Britton *et al*., 1997), and^{99m}Tc-Kanamycin (Roohi *et al*., 2006a). Several Technetium-99m based radio pharmaceuticals have also been presented for renal scintigraphy (Poropat *et al*., 1999; Kahn *et al*., 1994). These are ^{99m}Tc-Gluco-ene-diolate (^{99m}Tc-Sn-Gluco) and ^{99m}Tc-pyrolidinomethyl-tetracycline (^{99m}Tc-Sn-PMT). The different parameters are used to influence the binding of proteins, such as type of protein, charge of the agent, pH, and the amount of the compound in the blood (Yousif, 2010).

The lincomycin is an assorted group of protein inhibiting antimicrobials with activities similar to members of the macrolide group of antibiotics. Thus the macrolide group and the related lincosamides comprise antibiotics used for treatment of bacterial infections caused by gram positive organisms. Lincomycin was first produced from cultures of *Streptomyces lincolnensis.* In literature different procedures have been reported for lincomycin quantification, including microbiological assay method, gas chromatography (Luo *et al*., 1996) and HPLC (Olsovska *et al*., 2007).

In the present study, labeling protocol of Lincomycin with Technetium-99m was optimized. Biodistribution and scintigraphic studies of ^{99m}Tc-lincomycin in rats and rabbits respectively was also done.

MATERIALS AND METHODS

Material and methods

All the chemicals used were of analytical grade and purchased from Merck, Germany. Lincomycin hydrochloride was obtained from D. Watson Chemist, Islamabad, Pakistan. Rats (Sprague-Dawley) and rabbits were taken from National Institute of Health (NIH), Islamabad. *S. aureus* and *E. coli* bacteria's (ATCC 25923) were obtained from Department of biological sciences, Quaid-e-Azam University, Islamabad. The Animal Ethics Committee of the Institute has given the approval and a locally produced (n, f) PAKGEN ^{99}Mo/^{99m}Tc generator was used to obtain the Technetium-99m.

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Preparation of ^{99m}tc-Lincomycin

For preparation of ^{99m}Tc-Lincomycin, the amount of 0.1mg of Lincomycin hydrochloride was selected. The $SnCl_2.2H_2O$ (2-8 µg) was used as reducing agent at a pH of 5, which was the original pH of the mixture. Ascorbic acid (5 mg) was also used as antioxidant/stabilizer and pH of reaction mixture was varied to obtain maximum labeling efficiency of ^{99m}Tc-Lincomycin. Then ~370 MBq$^{99m}TcO_4^-$was injected into the vial containing 0.9% sodium Chloride solution.

Radiochemical purity determination

For radiochemical purity of ^{99m}Tc-Lincomycin, a 1-µL sample of the preparation was spotted on ITLC strips (Gelman, USA) and developed using 0.5M NaOH as the mobile phase. Watt man Paper No. 3 was developed using acetone as the mobile phase to quantify the free pertechnetate. The radio analysis of ^{99m}Tc-Lincomycin, free pertechnetate and hydrolyzedon chromatographic strips was quantified by a 2π Scanner (Berthold, Germany).

High performance liquid chromatography (HPLC) analysis

The ^{99m}Tc-Lincomycin was characterized by HPLC analysis using a D-200 Elite HPLC system with C-18 column (Alltech). The mobile phase used for HPLC analysis was consist of a mixture of acetonitrile (ACN) and0.02Msodium dihydrogen phosphate having ratio 850: 150 (v/v %) with the flow rate of 1mL/min. UV detector was used for the detection of lincomycin and spectrum was measured at 220nm wavelength.

Electrophoresis of ^{99m}Tc-lincomycin

Electrophoresis of^{99m}Tc-Lincomycin was studied by using Deluxe Electrophoresis Chamber (Gelman) System having phosphate buffer of pH 6.8 and the strip Whatman No.1 paper of 30cmplaced in the electrophoresis chamber. The drop of ^{99m}Tc-lincomycinwas poured on the strip and electrophoresis was performed for 40- 60 min at a voltage of 300v. Finally strip was scanned by using 2π scanner to detect charge on ^{99m}Tc-Lincomycin.

Lipophilicity test of ^{99m}Tc-Lincomycin

Lipophilicity was studied by mixing 1.5mL of ^{99m}Tc-Lincomycininto 2-3mL of carbon tetrachloride (CCl_4). The mixture was shaken vigorously for 20 min. The organic and aqueous layers were carefully separated and checked for the counts by using Ludlum γ-counter.

In-vitro Stability of ^{99m}Tc-Lincomycin

The ^{99m}Tc-Lincomycin stability was determined in human serum at 37ºC. Normal human serum (1.8mL) was mixed with 0.2mL of ^{99m}Tc-lincomycin, incubated for 30 min and 0.2mL aliquots were subjected to ITLC/paper chromatography for the evaluation of radio labeledlincomycin, hydrolyzed /reduced ^{99m}Tc and free $^{99m}TcO_4^-$.

In vitro cell binding studies

For *in-vitro* study of binding with cell, 0.1mL sodium phosphate buffer (Na-PB) having ~5MBq activity of Technetium-99m labeled lincomycin was putin a test tube and 0.01*M* acetic acid (0.8mL) with Na-phosphate buffer having the viable bacteria of nearly 1×10^8 was employed and was also incubated at a temperature of 4ºC for 1 hour. The mixture's centrifugation was carried out at a temperature of 4ºC for10minutes and after removing supernatant radio activity in the pallet containing bacteria was determined by a gamma-counter. For comparison purpose, the bacterial binding toTc-99m-Ascorbic acid was also performed. Same method was performed for *in vitro* binding study of ^{99m}Tc-Lincomycin with *E. coli* and ^{99m}Tc-Ascorbic acid with *S. aureus* and *E. coli.*

Infection/ inflammationin thigh muscle model

The volume of 0.2mL saline containing a turbid like suspension having 2×10^8 cfu *Staphylococcusaureus* for infection and Heat-Killed *S. aureus* for inflammation were injected into the Male Sprague-Dawley rat's left thigh muscle. Then the same 0.2mL volume of ^{99m}Tc-Lincomycin (~37 MBq) was injected through rat's tail vein after 24h and the activity present in whole blood was determined. The bacterial uptake of ^{99m}Tc-Lincomycin and other radio pharmaceuticals were quantified by the analysis of variance.

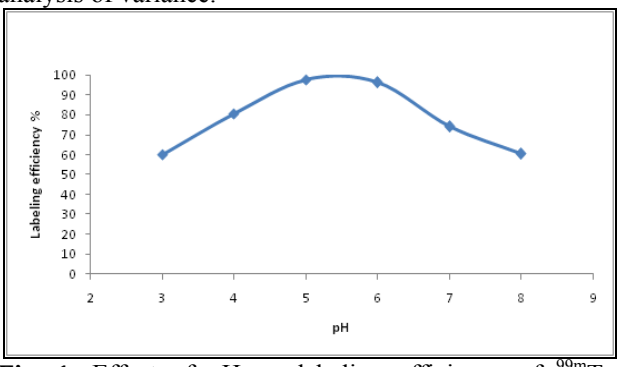

Fig. 1: Effect of pH on labeling efficiency of ^{99m}Tc-Lincomycin

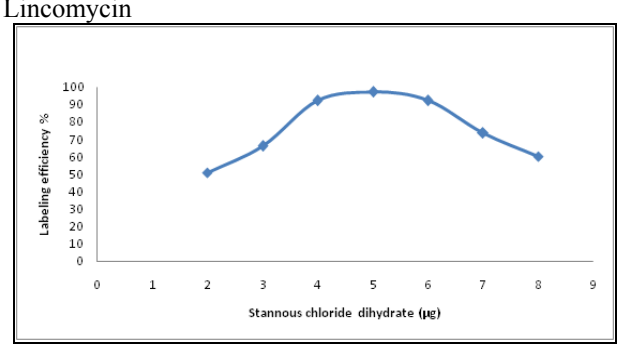

Fig. 2: Effect of amount of reducing agent on labeling efficiency of ^{99m}Tc-Lincomycin

Infection induction

For scintigraphy purpose, a volume of 0.6mL saline was injected; having 4×10^8colony-forming unit's viable *Staphylococcusaureus* in to the rabbit's left thigh muscle.

Scintigraphystudy of ^{99m}Tc-lincomycin

Siemens Integrated Orbiter Gamma Camera System linked to on line computer (Macintosh® Operating System 7.5 Software used on the ICON™ Workstation) was used. Diazepam (5mg) was injected into the right thigh muscle. The marginal ear vein of the rabbit was used for intravenous injection of 0.2mL saline having 15 MBq of Technetium-99m labeled Lincomycin. Immediately, after the intravenous injection, focus the rabbit's both thighs and dynamic acquisition was carried out for the duration of 120min. whereas, for biodistributional evaluation of Technetium-99m labeled Lincomycindynamic acquisition of whole body was also performed at 30 min, 1 hour and 4 hours after post injection.

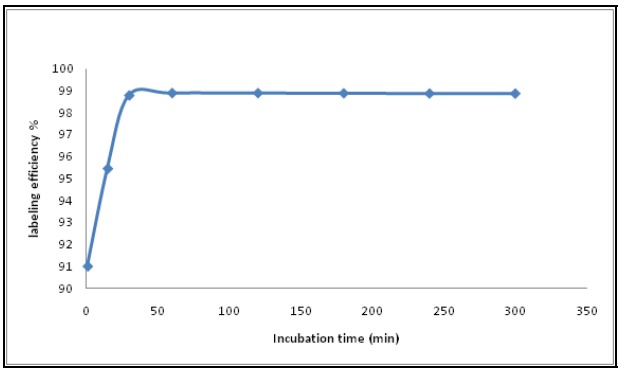

Fig. 3: Rate of complexation and stability of ^{99m}Tc-Lincomycin at room temperature

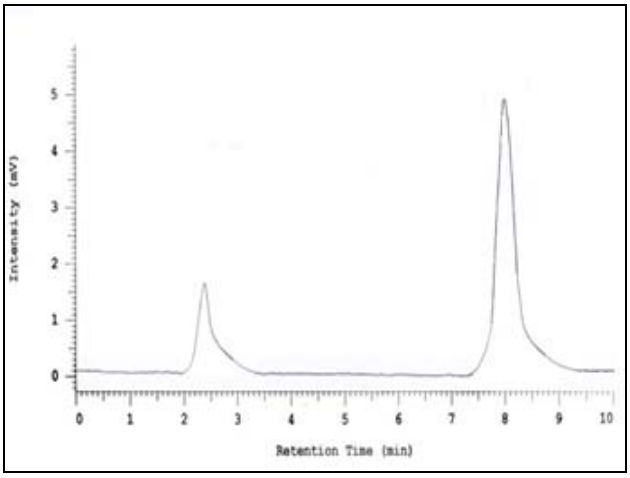

Fig. 4: HPLC chromatogram showing the percentage purity of Lincomycin

RESULTS

Labeling efficiency, stability and radiochemical quality control of Lincomycin with ^{99m}Tc was assessed by ascending paper chromatography technique in which acetone was used as the solvent, ^{99m}Tc-Lincomycin and hydrolyzed/reduced ^{99m}Tc remained at the origin while free $^{99m}TcO^{-4}$ moved towards the solvent front (R_f=1). While in ITLC-SG chromatography technique, 0.5 M NaOH was used as solvent, and reduced/hydrolyzed Technetium-99m was remained at the origin, while the ^{99m}Tc-Lincomycin and the free $^{99m}TcO^{-4}$ were moved in the direction of solvent front. The amount of ligand (Lincomycin) used in each experiment was 0.1mg. Regarding effect of pH, at low pH 3 and 4radio-labeling efficiency was less as 60% and 80% respectively, whereas, at 5 pH and 6 pH the radio-labeling efficiency of ^{99m}Tc-Lincomycin obtained to be >95% (fig. 1). At basic side, pH 8 the efficiency of labeling was decreased to 60%. The amount of stannous chloride was 4-6 µg which showed highest labeling yield; hence 5µg of reducing agent was used for further studies (fig. 2). The complexation of lincomycin withTechnetium-99m was a rapid process, but the stability of the complex deteriorated sharply. An antioxidant ascorbic acid was therefore added to stabilize ^{99m}Tc-Lincomycin and more than 98% labeling efficiency was achieved for 5 h with the addition of ~5 mg ascorbic acid (fig. 3). The final formulation contained lincomycin (0.1 mg), 5 µg of $SnCl_2.2H_2O$, 5 mg ascorbic acid, pH 5, 370 MBq ^{99m}Tc in ~ 1.5 mL solution (total volume). The results of HPLC showed that ligand purity was greater than 98% (fig. 4). The inactive lincomycin and ^{99m}Tc-lincomycin retention time was 7.5 minutes. fig. 5 indicates the labeling efficiency of ~98%.

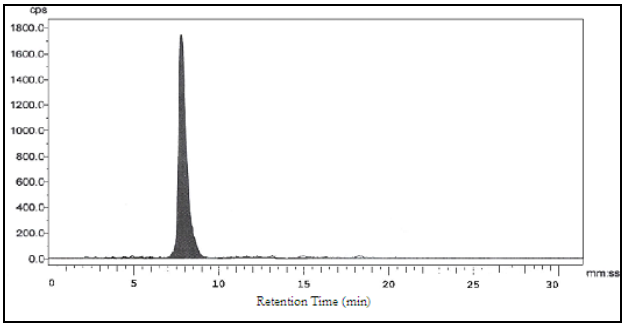

Fig. 5: HPLC analysis of lincomycin labeled with ^{99m}Tc Shows single species of complex

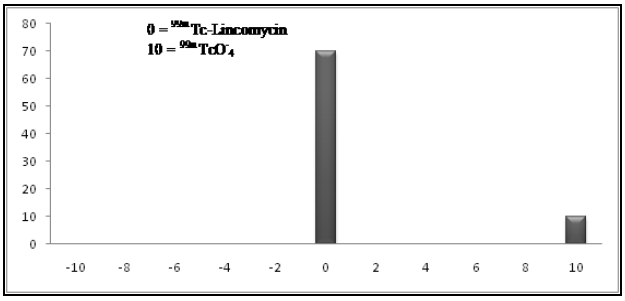

Fig. 6: Electrophoresis of ^{99m}Tc-Lincomycin

The results of electrophoresis showed that the ligand was neutral in nature and the free pertechnetate was moved towards anode after 1 hour (fig. 6). The lipophilicity test indicates that organic layer has >98% ^{99m}Tc-Lincomycin which indicates that ^{99m}Tc-Lincomycin was lipophilic in nature. During incubation in human serum, ^{99m}Tc-lincomycin was quite stable as determined by

paper/ITLC-SG. Up to 92% labeling was found at 24 h of incubation at 37ºC and there was slightly increase in reduced/hydrolyzed and free ^{99m}Tc (table 1). The *in vitro* binding of bacteria to ^{99m}Tc-lincomycin was comparable with ^{99m}Tc-Ascorbic acid. Binding of ^{99m}Tc-lincomycin was in the range of 95-98% for *S. aureus* and 50-89% for *E. coli* (table 2), while binding of ^{99m}Tc-Ascorbic acid was <5% (table 3). Table 4 depicts the biodistribution data in rats.

Table 1: *In vitro* stability of ^{99m}Tc-Lincomycin in normal human serum, n=3

Incubation time/H	^{99m}Tc-lincomycin	Free pertechnetate	Colloid
1	96.8±2.2	3.1±0.6	1.0±0.4
2	93.7±2.1	3.3±0.8	2.9±0.6
4	92.6±2.0	3.4±1.1	3.9±0.7
24	92.0±1.9	3.8±1.4	4.2±0.9

Table 5 showed that the normal thigh and infected thigh radioactivity was obtained at 1h, 4h and 24h after ^{99m}Tc-Lincomycin administration. In these time intervals, the target-to-non target ratios of radioactivity was indicated that infection induced by *Staphylococcus aureus* has significant binding affinity, while the binding affinity of ^{99m}Tc-Lincomycin to the inflammation induced thigh with Heat-killed *S. aureus* and normal thigh was not so significant. The target/non target ratio reached 1.85, 2.33 and 3 at 1h, 4h and 24h, respectively. The infected rabbit's whole body images at 30 min, 1 hour, and4 hours after post administration of ^{99m}Tc-Lincomycin are shown in fig. 7, which shows the maximum accumulation of labeled Lincomycin at infection site in left thigh as highlighted by a circle.

In the rabbit's left thigh, the infection induced by *Staphylococcus aureus* was clearly visualized as the area of higher radiotracer accumulation immediately after the injected labeled Lincomycin (fig. 8).

Table 2: *In vitro* binding (%) of the ^{99m}Tc-Lincomycin to viable *S. aureus* and *E. coli*, n=3

^{99m}Tc-Lincomycin	*S. aureus*			*E. colli*		
	1 Hour	4 Hours	24 Hours	1 Hour	4 Hours	24 Hours
10 µg	98.55±1.1	98.88±1.2	97.77±1.3	89.56±2.2	83.65±3.5	85.66±4.6
50 µg	96.87±1.4	95.77±1.5	98.77±1.3	64.49±3.2	62.17±3.5	63.58±5.2
100 µg	95.69±1.5	97.89±1.4	95.88±1.2	50.84±3.3	50.49±4.8	50.32±3.3

Table 3: *In vitro* binding (%) of the ^{99m}Tc-Ascorbic acid to viable *S. aureus* and *E. coli*, n=3

^{99m}Tc-Ascorbic acid	*S. aureus*			*E. coli*		
	1 h	4 h	24 h	1 h	4 h	24 h
10 µg	0.16±0.04	0.10±0.05	0.08±0.04	0.25±0.09	0.17±0.05	0.08±0.03
50 µg	0.37±0.08	0.25±0.05	0.12±0.05	0.49±0.11	0.27±0.09	0.14±0.08
100 µg	3.9±0.55	3.2±0.41	1.8±0.45	4.5±0.51	2.8±0.66	1.2±0.31

Table 4: Biodistribution data in percent injected dose per gram organ/tissue for ^{99m}Tc-Lincomycin at 1, 4 and 24 hours after intravenous administration in *S. aureus* infected vs. Heat-Killed *S. aureus* inflamed rats, n=3

Organ	Percentage of injected dose per gram tissue					
	S. aureus			Heat-Killed *S. aureus*		
	1 h	4 h	24 h	1 h	4 h	24 h
Liver	3.65±0.23	5.66±0.40	1.47±0.21	4.84±0.45	2.98±0.34	1.54±0.23
Spleen	0.96±0.04	0.58±0.03	0.22±0.02	2.57±0.42	1.89±0.032	0.78±0.021
Stomach	0.99±0.03	0.96±0.03	0.43±0.01	1.54±0.07	0.78±0.03	0.36±0.03
Intestine	1.09±0.12	2.22±0.41	1.63±0.21	1.71±0.12	0.79±0.12	0.42±0.07
Lungs	9.5±0.52	3.61±0.27	0.63±0.06	9.11±0.59	7.21±0.47	3.68±0.31
Kidney	3.95±0.21	2.85±0.13	0.88±0.09	14.11±0.98	9.56±0.89	4.98±0.87
Femur	1.48±0.15	2.05±0.21	0.68±0.06	1.86±0.21	0.92±0.09	0.59±0.05
Infec-Femur	1.97±0.21	2.99±0.30	1.24±0.19	1.81±0.26	1.11±0.20	0.88±0.07
Urine	0.03±0.01	0.14±0.07	6.93±1.58	4.26±1.12	2.96±0.51	6.99±1.10
Bladder	8.0±1.5	2.05±0.55	0.62±0.12	11.14±1.8	8.34±2.75	4.33±1.11
Heart	2.00±0.18	1.88±0.23	0.85±0.21	4.14±0.45	3.05±0.34	1.14±0.23
Brain	1.58±0.21	1.12±0.20	0.81±0.15	1.02±0.23	0.75±0.17	0.35±0.11
Blood	3.45±0.42	3.64±0.51	3.79±0.45	3.00±0.51	3.21±0.42	3.48±0.48
Body	1.33±0.21	3.21±0.41	0.95±0.12	2.23±0.44	2.85±0.46	1.66±0.23

Table 5: Target-to-non target ratios of ^{99m}Tc-Lincomycin in thigh muscle-infected rats and thigh muscle-inflamed rats at 1, 4 and 24 hours after injection, n=3

Tissue	*S. aureus*			Heat-Killed *S. aureus*		
	1 h	4 h	24 h	1 h	4 h	24 h
Target %	65±3	70±4	75±4	43±2	37±2	35±2
Non target %	35±2	30±2	25±2	57±2	63±3	65±3

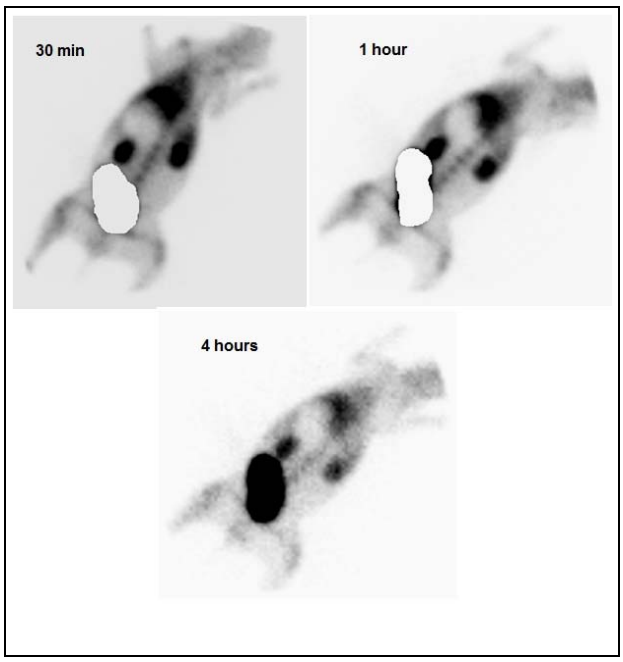

Fig. 7: Whole body gamma camera images of infected rabbits injected with ^{99m}Tc-Lincomycin at 0.5h, 1h and 4h

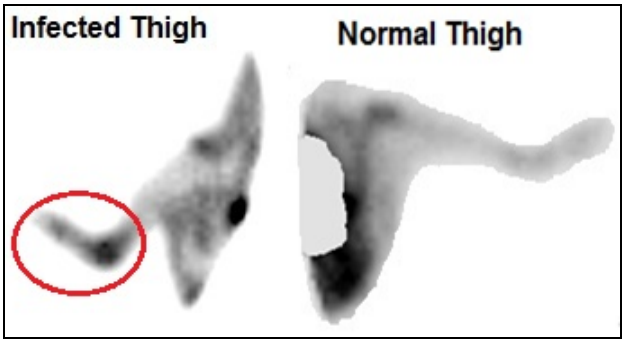

Fig. 8: *S. aureus* infected left thigh and normal right thigh of rabbit.

DISCUSSION

Radiochemical purity and stability of ^{99m}Tc-lincomycin were assessed by thin layer chromatographic technique and reversed phase high performance liquid chromatography. The comparison was made to estimate the influence of ^{99m}Tc-Ascorbic acid impurity, which may be present in the preparation. Varying amounts of lincomycin (10 to100µg) showed same binding efficiency with *S. aureus*, whereas higher concentration of lincomycin showed low efficiency for *E. coli*. The distribution of Technetium-99m labeled lincomycin was presented as% of dose injected per organ with the bacterial infections and induced inflammations. The ^{99m}Tc-Lincomycin was quickly distributed, although liver, heart and intestine shows significant uptake due to the high lipophilicity of the labeled compound. Bone uptake of ^{99m}Tc-Lincomycin in rat was quite high, but inferior to ^{99m}Tc-MDP (Heggli *et al*., 1988).

The infection in left thigh of the rabbit is clearly visible at 30 min, 1 hour and 4 hours ^{99m}Tc-Lincomycin administration, while at 24 hours, the activity in urinary bladder was very high, due to which the infection sites was not so clearly visualize as compared to the 30 min, 1hour and 4 hour images as well.

CONCLUSIONS

This work describes the direct labeling method for preparation of ^{99m}Tc-Lincomycin with high radiochemical purity. The labeling efficiency (≥98%) and stability of ^{99m}Tc-Lincomycin complex was quite high. The radioactive preparation of ^{99m}Tc-Lincomycin found to be capable of localizing the *Staphylococcus aureus* induced bacterial infection in the animal models, also from the results, it is concluded that the ^{99m}Tc-Lincomycinmay be used as infection imaging agent.

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REFERENCES

Bhatnagar A, Lahoti D, Singh AK, Shankar LR, Sharma B and Singh T (1995). Scintigraphic diagnosis of protein losing enteropathy using ^{99m}Tc-dextran. *Clin Nucl Med,* **20**: 1070-1073.

Bokhari T H, Mushtaq A, Hina S, Bokhari I H, Yousaf M, Ahmad I, Rasool S, Zubair M, Naqvi AR and Zahoor A F (2012). Concentration of medically interesting ^{99m}Tc-

pertechnetate for diagnostic radio pharmaceuticals. *J. Chem. Soc. Pak.,* **34**: 462.

Britton K, Vinjamuri S, Hall A, Solanki K, Siraj, Bomanji J and Das S (1997). Clinical evaluation of ^{99m}Tc infection for the localisation of bacterial infection. *Eur J. Nucl. Med ,* **24**: 553-556.

Buscombe JR, Lui D, Ensing G, de Jong R and Ell PJ (1990). ^{99m}Tc-human immunoglobulin (HIG) first results of a new agent for the localization of infection and inflammation. *Eur. J. Nucl. Med.,* **16**: 649-655.

Degirmenci B, Kilinc O, Cirak K A, Capa G, Akpinar O, Halilcolar H, Durak H, Akkoclu A, Derebek E and Ucan ES (1998). Technetium-99m-tetrofosmin scintigraphy in pulmonary tuberculosis. *J. Nucl Med.,* **39**: 2116.

Essouissi I, Ghali W, Saied N M and Saidi M (2010). Synthesis and evaluation of ^{99m}Tc-*N*-sulfanilamide ferrocene carboxamide as bacterial infections detector. *Nucl. Med. and Bio.,* **37**: 821-829.

Faheem A, Bokhari T, Roohi S and Mushtaq A (2012). Direct labeling of doxorubicin with technetium-99m: Its optimization, characterization and quality control *J. Radioanal. Nucl. Chem.,* **293**: 303.

Faheem AR, Bokhari TH, Roohi R, Mushtaq A and Sohaib M (2013). ^{99m}Tc-Daunorubicin a potential brain imaging and theranostic agent: Synthesis, quality control, characterization, biodistribution and scintigraphy. *Nucl. Med. and Bio.,* **40**: 148.

Gallagher H, Ramsay S C, Barnes J, Maggs J, Cassidy N and Ketheesan N (2006). Neutrophil labeling with [^{99m}Tc]-technetium stannous colloid is complement receptor 3-mediated and increases the neutrophil priming response to lipopolysaccharide. *Nucl. Med. and Bio.,* **33**: 433-439.

Heggli D E, Franco P and Norbygaard E (1988). Differences in biodistribution in rats injected with ^{99m}Tc-MDP preparations with different stabilizing agents. *Eur. J. of Nucl. Med.,* **14**: 105.

Luo W, Yin B, Ang CY, Rushing L and Thompson HC (1996). Determination of erythromycin, clarithromycin, roxithromycin and azithromycin in plasma by high-performance liquid chromatography with amperometric detection. *J. Chromat. B.,* **687**: 405.

Melendez-Alafort L, Rodriguez-Cortes J, Ferro-Flores G, Arteaga De Murphy C, Herrera-Rodriguez R, Mitsoura E and Martinez-Duncker C (2004). Biokinetics of ^{99m}Tc-UBI 29-41 in humans. *Nucl. Med. and Bio.,* **31**: 373-379.

Olsovska J, Jelinkova M T, Man P, Koberska M T, Janata JA and Flieger M (2007). High-throughput quantification of lincomycin traces in fermentation broth of genetically modified Streptomyces spp. Comparison of ultra-performance liquid chromatography and high-performance liquid chromatography with UV detection. *J. Chromat. A.,* **1139**: 214-220.

Onsel C, Sonmezoglu K, Camsari G, Atay S, Cetin S, Erdil YT, Uslu I, Uzun A, Kanmaz B and Sayman HB (1996). Technetium-99m-MIBI Scintigraphy in Pulmonary Tuberculosis. *J. Nucl. Med.,* **37**: 233-238.

Passamonte PM, Seger RM, Holmes RA and Hurst DJ (1983). Technetium-99m glucoheptonate imaging in lung cancer and benign lung diseases: Concise communication. *J. Nucl. Med.,* **24**: 997.

Roohi S, Mushtaq A, Jehangir M and Malik S (2006). Synthesis, quality control and biodistribution of ^{99m}Tc-Kanamycin. *J. Radioanal. Nucl. Chem.,* **267**: 561-566.

Roohi S, Mushtaq A, Jehangir M and Malik SA (2006). Direct labeling of isoniazid with technetium-99m for diagnosis of tuberculosis. *Radiochimica. Acta.,* **94**: 147-152.

Kahn D, Ben-Haims S, Bushneel DL and Kirchner PTM (1994). Captopril-enhanced Tc99m-MAG3 renal scintigraphy in subjects with suspected reno vascular hypertension *J. Nucl. Med.*, **15**: 515.

Poropat MMD, Batnick DMD, Nizic LJ, Miloseric D, Votava-Riaic A, Tezak S, Vrljica K and Medvedec M (1999). Tc-99m DTPA renal Scientigraphy using Deconvolution analysis with six functional images of the mean time of evaluate acute pyelonephritis. *Clin. Nucl. Med.,* **24**: 120.

Yousif FS (2010). *In vivo* and *in vitro* ^{99m}Tc-protein bound of renal agents: ^{99m}Tc-prylidinomethylte-tracycline (^{99m}Tc-Sn-Pmt) and ^{99m}Tc-Sn-Gluco-ene-diolate (^{99m}Tc-Gluco). *J. Saudi Chem. Soc.,* **14**: 139-145.