Synthesis, optimization and biological evaluation of ^{99m}Tc-digoxin as possible cardiac imaging agent

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Abstract: Heart imaging radiopharmaceuticals could improve the diagnostic value of routine heart scanning for detecting cardiac disorders. The aim of the study was to prepare high radiochemical purity ^{99m}Tc-Digoxin in a yield of about 98%. The optimal conditions for labelling were as follows: 100µg of Digoxin, 2µg of SnCl2·2H2O, room temperature (25±1°C), reaction retention time of 30 min at pH 7. Under these conditions, the radiochemical yield of ^{99m}Tc-Digoxin reaches 98%. *In vivo* bio distribution was performed in normal Swiss Albino mice at different time intervals after administration of ^{99m}Tc-Digoxin.Scintigraphic study of ^{99m}Tc-Digoxin was performed in rabbits. The heart uptake of ^{99m} Tc-Digoxin was sufficiently high and thus may be a potential myocardial imaging radiopharmaceutical applicable in cardiology.

Keywords: ^{99m}Tc-Digoxin, chromatographic analysis, Bio distribution, myocardial imaging.

INTRODUCTION

Myocardial perfusion imaging employs evaluation of regional abnormalities in coronary artery blood flow and determines their physiological relevance to myocardial function (Udo *et al*., 2003; Thomas *et al*., 2008; Higley *et al*., 1993; Gibbons, 2000; Henzlova *et al*., 2006., Morrison *et al*., 2010).). The development of radiotracers labelled pharmaceuticals for cardiology is an important discipline and several radiotracers have been developed that show promising role in the cardiac disorders management (Renata *et al*., 2012). Technetium-99m is mostly used in all radio pharmacy centres where radiopharmaceuticals are formulated for imaging, due to its suitable physical and chemical characteristics, inexpensive isotope cost and availability. The technetium-99m based myocardial agents were used widely and recently received a great attention (Jurisson *et al*., 1993; Jones, 1995; Yang *et al*., 2003). For myocardial perfusion imaging ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI have been approved as commercial products (Banerjee *et al*., 2001). Various radiopharmaceuticals such as [^{99m}TcN(PNP5)(DMCHDTC)]$^{+}$ complex (Zhang *et al*., 2009), ^{99m}Tc-Losartan (Ibrahim *et al*., 2013), [^{99m}Tc/Re(CO)3]$^{+}$ (Zeng *et al*., 2014), ^{99m}Tc- Dextran (Eberhard *et al*., 1982), ^{186}Re-MIBI (Talaat *et al*., 2013), ^{125}I-Metoprolol (Ibrahim *et al*., 2010), thallium Tl 201 chloride (Baggish *et al*., 2008), Radio iodinated digoxin derivative (Fujibayashi *et al*., 1992), ^{67}Ga labelled digoxin derivatives (Fujibayashi *et al*., 1993), ^{99m}Tc-labelled conjugates of ouabagenin (Debnath *et al.,* 2006) have been developed for cardiac imaging. Digoxin is a compound belongs to the family of drugs known as cardiac glycosides which has very important role in congestive heart failure therapy. The compound Digitalis causes increase in intracellular Ca^{2+} activity in heart cells which is the basis of inotropic and arrhythmogenic effects of the drugs (Eisner *et al*., 1991)

In this study we developed a simple method for ^{99m}Tc labeling of Digoxin. Labelling efficiency, optimization, radiochemical purity, stability and characterization were also studied. The bio-evaluation of ^{99m}Tc-Digoxin for imaging in animal was performed by different *in vitro* and *in vivo* assays followed by scintigraphy.

MATERIALS AND METHODS

All chemicals used were of Analytical grade and obtained from E. Merck (Germany) and no purification of chemicals was performed. Digoxin was purchased from Well come Pakistan; Ltd. SnCl2.2H2O (reducing agent) was purchased from Sigma, USA. Technetium-99m was achieved from a locally produced fission based PAKGEN ^{99}Mo/^{99m}Tc generator. Swiss Webster mice, weighing approximately 28 g were obtained from the Animal House at School of Biological Sciences (SBS), University of the Punjab, Lahore, Pakistan. Rabbits were purchased from NIH, Islamabad. Tissue and organ radioactivity was measured with a γ-counter (Ludlum model 261). For imaging studies of rabbits Gamma scintillation camera was used. Animal experiments were performed according to rules approved by Institutional Animal Ethics committee. All the chemicals of mostly analytical grade were used in this study.

Labeling procedure of ^{99m}Tc-Digoxin

The labeling was performed by adding 100µg Digoxin and 2µg of reducing agent (SnCl2.2H2O) at pH 7 for

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thirty minutes. At the end ~296 MBq $^{99m}TcO^{-}4$ in saline was added in vial. The volume of reaction mixture used in all experiments was 1±0.2mL at room temperature.

Factors effecting percentage of labeling yield

Experiments were performed at different concentrations of Digoxin (50-250µg), SnCl2 (2-10µg), and the pH was studied from 3 to 11. The reaction mixture at different time intervals (30min- 5hrs) was kept at room temperature (25±1°C) to observe the stability or retention time. The optimum time of each factor was determined by trial and error method. The experiment was performed by keeping all the factors constant except the one which is under study until the optimum conditions were determined.

Determination of radiochemical purity

The radiochemical purity of ^{99m}Tc- Digoxin was determined by chromatographic techniques. Reaction mixture was spotted on chromatography strips (ITLC-SG, Whatman No. 3 paper). Free $^{99m}TcO^{-}4$ in the preparation was determined by the use of acetone as mobile phase. Reduced or hydrolyzed technetium was determined by 0.5M NaOH as a mobile phase to differentiate between reduced colloids which stick near the point of spotting and free and complex both move towards the front of chromatogram. After completely drying these strips, the activity was determined with the help of 2π scanner (Berthold, Germany).

Electrophoresis of ^{99m}Tc- Digoxin

Electrophoresis is done by the application of radioactive sample spotted on a piece of Whatman1 chromatography paper soaked in a 0.05M Phosphate buffer of pH 7 and applying an appropriate voltage across the paper for a period of 1hour. The radioactivity distribution on strip was checked after drying with the help of 2π scanner. The components of the sample move along the paper depending on their charge and ionic mobility.

High Performance Liquid Chromatography

HPLC was used for checking the radiochemical purity of the ^{99m}Tc-Digoxin. The HPLCof^{99m}Tc-Digoxin was carried out by means of D-200 Elite HPLC system. HPLC was performed on the final preparation using a C-18 column as stationary phase and mixture containing methanol and Phosphate buffer as stationary phase in the ratio 90:10 [v/v %]. Mobile phase flow rate was adjusted up to 1 mL per minute. During experiment, UV detector at wavelength of 254 nm was used for the purpose of detection.

In vitro stability in normal human serum

The normal human serum (1.8mL) and ^{99m}Tc-Digoxin (0.2 mL) was mixed and incubated at 25ºC. The aliquots of 0.2 mL were withdrawn at different time intervals up to 24 hours during the incubation and subjected to HPLC and Instant Thin layer chromatographic analysis for determining radiochemical purity of ^{99m}Tc-Digoxin and reduced/hydrolysed ^{99m}Tc and for free $^{99m}TcO^{-4}$.

Biodistribution study of ^{99m}Tc-Digoxin

A Solution of ^{99m}Tc-Digoxin (0.3mL, 37MBq) was injected to normal Swiss Albino mice (25-30g) through tail vein. After definite intervals of time (15min, 30min, 1h and 4h), the mice were sacrificed. Organs or tissues of interest were removed, weighed, counted for activity with the help of gamma counter and corrections were done for the background radiation. The results were calculated as the % of injected dose per gram tissue. Three mice were sacrificed on each set of experiment.

Scintigraphic study of ^{99m}Tc- digoxin

The imaging of ^{99m}Tc-Digoxin was done by Scintigraphic images of rabbit recorded at different time points. 0.2mL technetium-99m labeled digoxin was injected in rabbit through marginal ear vein and scanning was performed at 2 min, 5min, 10min, 20min, 30min and 4h after injection for the biological distribution evaluation of the medical radiotracer. A single headed Siemens Integrated ORBITER Gamma Camera System under low energy conditions and high resolution.

RESULTS

Labelling of ^{99m}Tc-Digoxin

The direct labelling methodology of digoxin with technetium-99m was evaluated. The structural formula of digoxin (M.W. =708.95) is given in fig. 1. In paper chromatographic study acetone was used as the solvent and Whatman No. 3 paper was used as the stationary phase. With acetone as mobile phase, free $^{99m}TcO^{-4}$ moved with solvent front, whereas the labeled and reduced colloids remain near spotting point. In ITLC-SG chromatographic technique with 0.5M NaOH as a solvent, the reduced or colloids persisted at the site of spotting and labeled and free pertechnetate moved to front of chromatogram (fig 2, 3). The optimum parameters affecting the labeling yield were accessed. The effect of amount of reducing agent is summarized in fig 4. The highest labeling efficiency was achieved at 2 µg of SnCl2.2H2O. The labeling was decreased with the increase in amount of SnCl2.2H2O from 2to 10 µg. The effect of pH on labeling yield of ^{99m}Tc-Digoxin is shown in fig. 5. At low pH (2-5) the labeling efficiency was to 82-95% which increased to 98 % at pH 7. In basic media 9 the labeling efficiency was decreased to a large extent. Therefore pH 7 was preferred for future experiments. The complexation of ^{99m}Tc with Digoxin is very quick and within minutes maximum labeling efficiency is achieved. At 100µg ligand there is found maximum labeling efficiency for up to 150µg but as the amount is increased significantly beyond this limit the labeling efficiency was decreased (fig. 6) thus 100µg of ^{99m}Tc-Digoxin was fixed to obtain the maximum

labeling yield.The labeling of ^{99m}Tc with Digoxin was 94% at 10 minutes. Labeling yield increased to 98% at 30 minutes of retention time and after this the amount of free ^{99m}Tc increased and labelling was decreased. Graphical representation of these results is depicted in fig. 7.

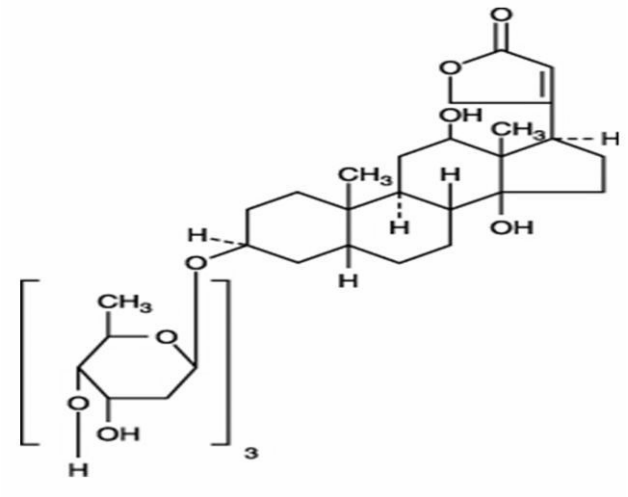

Fig. 1: Structure of Digoxin

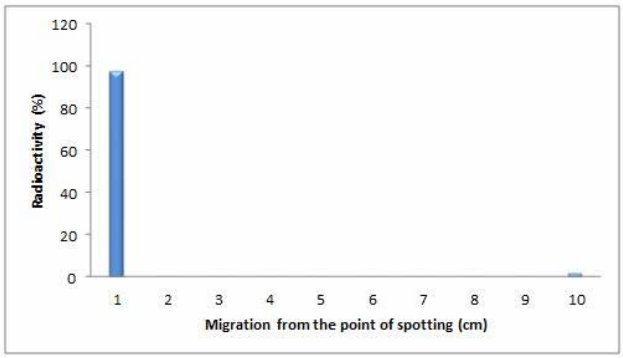

Fig. 2: Radio chromatogram showing positions of Red/hyd ^{199m}Tc, ^{99m}Tc-Digoxin Complex and free ^{99m}Tc (Stationary phase = ITLC-SG, Solvent system = Acetone)

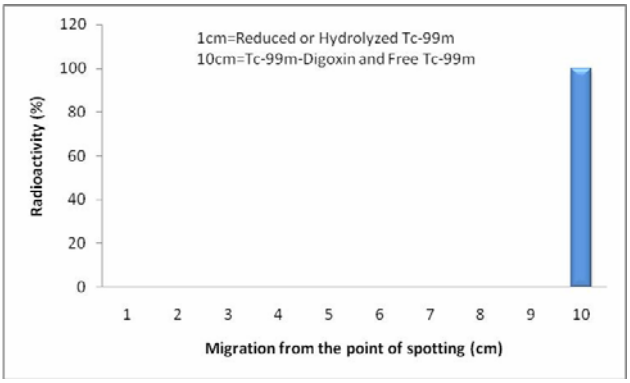

Fig. 3: Radio chromatogram showing positions of Red/hyd ^{99m}Tc, ^{99m}Tc-Digoxin Complex and free ^{99m}Tc (Stationary phase = ITLC-SG, Solvent system = 0.9% NaCl)

Electrophoresis and HPLC analyses

The paper electrophoresis illustrated that ^{99m}Tc-Digoxin ligand is polar in nature. The migration of labeled compound was towards cathode as shown by results in fig. 8. HPLC results of inactive ligands illustrates that the ligand purity was about 90%. The HPLC analysis of ^{99m}Tc-Digoxin demonstrates that about 99% of the compound binds with Tc-99m (fig 9, 10).

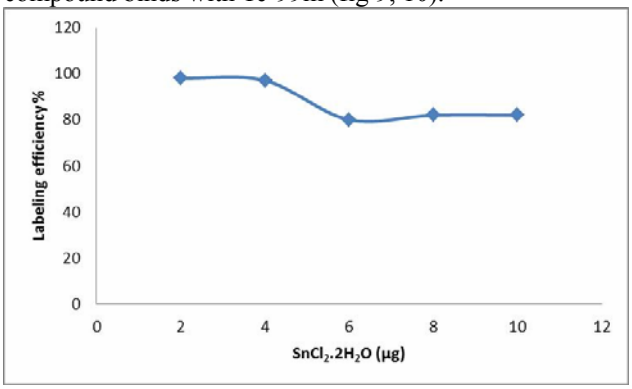

Fig. 4: Effect of SnCl2.2H2O amount on labelling efficiency of ^{99m}Tc- Digoxin

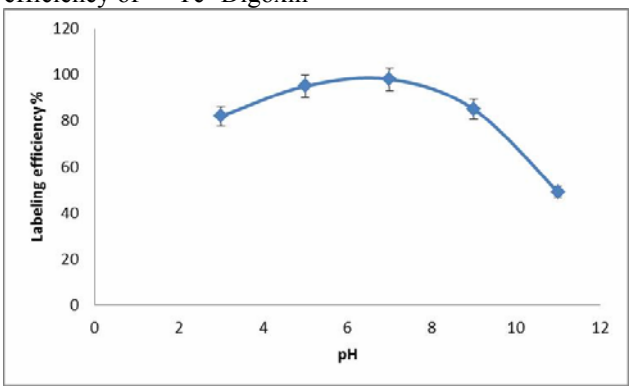

Fig. 5: Effect of pH of reaction medium on labelling efficiency of ^{99m}Tc-Digoxin

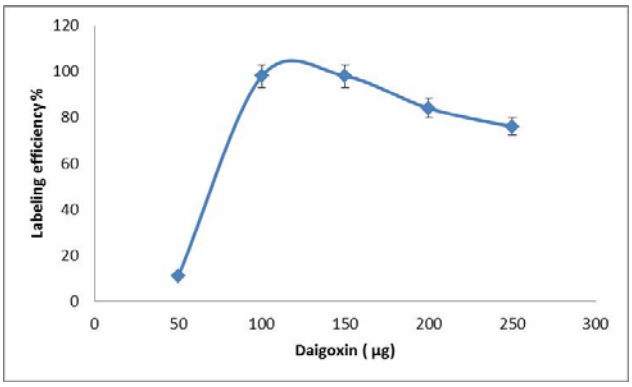

Fig. 6: Effect of amount of ligand (digoxin, µg) on the labelling efficiency of ^{99m}Tc-Digoxin

In vitro stability

When the prepared radiotracer was incubated with human serum at 25ºC, increase in reduced/hydrolyzed ^{99m}Tc colloid formation was observed up to 24 hours. At 24 hours about 60% stability was observed (fig. 11). Instant thin layer chromatography study of the human serum revealed that the ^{99m}Tc-Digoxin showed good stability during incubation at room temperature with human serum.

In vivo bio distribution and SPECT study

The *in vivo* behaviour of ^{99m}Tc-Digoxin was studied in mice at 15min, 30min, 1h and 4h post injection intravenously. ^{99m}Tc-Digoxin was distributed rapidly and significant uptake was seen in heart while good extent of radiopharmaceutical was also seen in stomach (table 1). ^{99m}Tc-Digoxin was further selected for the scintigraphy in order to better understand the results depicted earlier. fig. 12 shows the whole body imaging of rabbit at different time intervals after intravenous post administration.

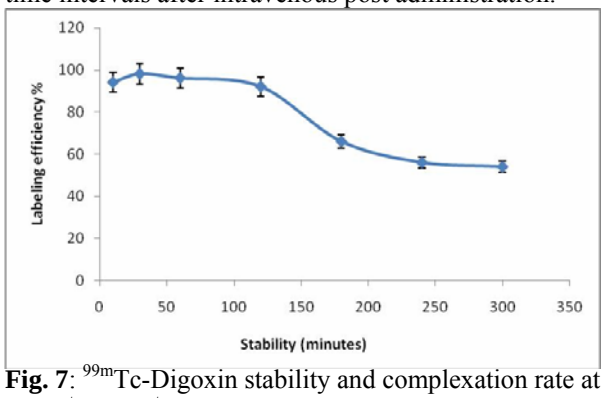

Fig. 7: ^{99m}Tc-Digoxin stability and complexation rate at room temperature

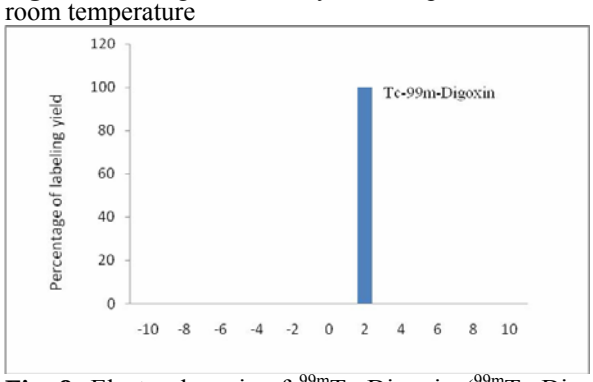

Fig. 8: Electrophoresis of ^{99m}Tc-Digoxin (^{99m}Tc-Digoxin at 2cm)

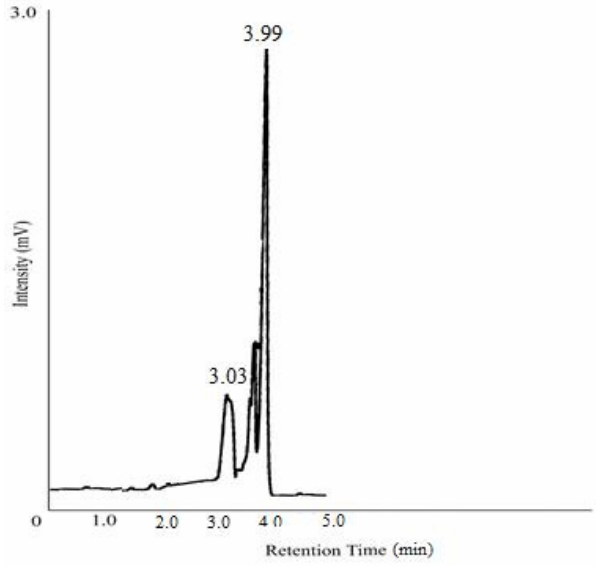

Fig. 9: HPLC analysis (UV detector) showing the purity of Digoxin

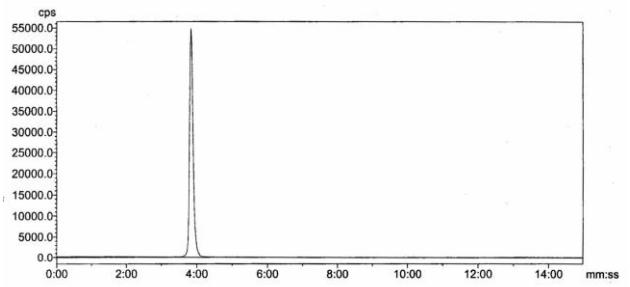

Fig. 10: HPLC analysis shows the percentage labeling of Digoxin with Tc-99m

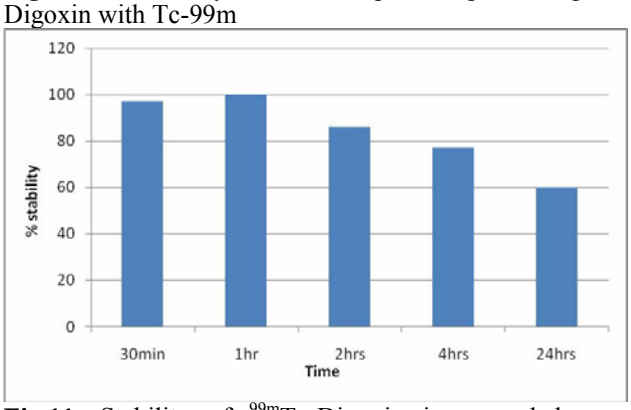

Fig.11: Stability of ^{99m}Tc-Digoxin in normal human serum at different time intervals

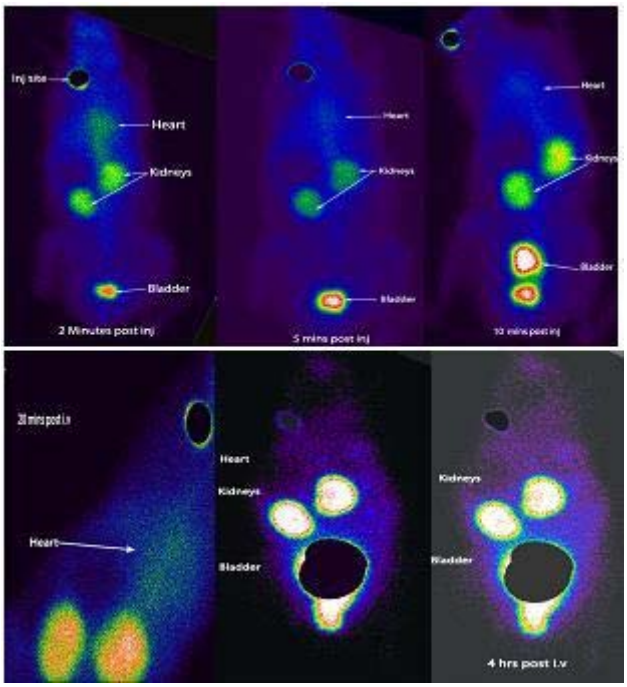

Fig. 12: Whole body gamma camera image of rabbit injected with ^{99m}Tc-Digoxin at different time intervals after intravenous post administration

DISCUSSION

Digoxin labelled with ^{99m}Tc with the help of stannous chloride (reducing agent) presented a good labelling efficiency. The final formulation for ^{99m}Tc-Digoxin was:

Table 1: Biodistribution of ^{99m}Tc-Digoxin in normal mice tissues (% injected dose per gram organ) at 30 min, 1h, 4h and 24h after intravenous administration (n=3), (mean ± SD).

Organ	Percentage of Injected Dose per Gram organ			
	15 min	30 min	1 hr	4 hr
Heart	4.1±0.11	9.5±0.1	2.28±0.03	0.23±0.01
Liver	2.31±0.09	1.02±0.1	1.02±0.05	2.14±0.02
Spleen	1.85±0.05	0.6±0.03	0.14±0.1	0.15±0.04
Lungs	2.5±0.03	4.42±0.1	0.97±0.5	0.33±0.07
Intestine	1.43±0.01	1.01±0.02	1.8±0.05	1.7±0.03
Kidney	2.4±0.07	3.12±0.09	3±0.1	2.01±0.1
Blood	2.3±0.09	3.51±0.01	0.31±0.04	0.65±0.02
Brain	0.32±0.05	0.02±0.05	0.26±0.1	1.08±0.1
Urine	52±0.08	50±0.07	52.3±0.10	51.3±0.12
Femur	0.34±0.05	0.6±0.02	0.31±0.1	0.7±0.3
Carcass	1.48±0.1	2.3±0.05	0.74±0.07	0.78±0.05

Digoxin 100µg; SnCl2.2H2O 2µg; pH ~7; ^{99m}Tc 296 MBq and 1.5mL of reaction mixture volume. The first and second sugars of digoxin at C-3 position were crucial for myocardial accumulation, but the third sugar having two hydroxyl groups could be a site capable of chelating ability for technetium-99m labeling. *In vitro* study on the stability of ^{99m}Tc-Digoxin demonstrated that human serum contained approximately 100% of as an intact complex within 60 min after introducing it to the serum. Biodistribution studies in mice indicate that complex exhibits a tissue distribution of ^{99m}Tc-Digoxin with rapid extraction from the blood into perfused myocardium, liver and kidneys. The percentage dose per gram organ of medically interesting radiotracer Tc-99m labelled with digoxin in heart was 4.1±0.11, 9.5±0.1, 2.28±0.03, 0.23±0.01 at 15min, 30min, 1hr and 4hr after intravenous administration. ^{99m}Tc-Digoxin was found to have a significant heart uptake as presented in table 1, suggesting it may be a potential myocardial imaging agent. The uptake of ^{99m}Tc-Digoxin is comparable to other ^{99m}Tc-labeled myocardial imaging agents like ^{99m}TcN (DMCHDTC)2 (Ibrahim *et al.,* 2010), ^{99m}TcN (PNP5) (DMCHDTC)]+ (Baggish et al., 2008) and ^{99m}Tc-Losartan (Ibrahim *et al*., 2013). The blood clearance was fast while activity remained in liver and kidneys. The digoxin clearance involves both metabolic and renal clearance from the body. The stomach uptake of the radiotracer (5-7%) was also significant after renal uptake and that may be due to the reason that digoxin is metabolized in the stomach by gastric acid in addition to renal clearance from body. Brain uptake of ^{99m}Tc-Digoxin was much lower because it is unable to cross the blood brain barrier due to its cationic nature. The significant uptake of ^{99m}Tc-Digoxin by heart with good retention time and high target/non-target ratio are the excellent characteristic for its application as a possible cardiac imaging agent.

CONCLUSION

During this study, ^{99m}Tc-Digoxin was prepared using 100 µg of digoxin and 2µg reducing agent (SnCl2.2H2O) at pH 7 and reaction time of 30 min. Labelling efficiency of ^{99m}Tc-Digoxin monitored by standard chromatographic technique was ~98%. The electrophoresis analysis of ^{99m}Tc-Digoxin showed its polar nature. HPLC represented the purity of ligand and ^{99m}Tc-Digoxin. The ^{99m}Tc-Digoxin complex was quite stable in human serum. Biodistribution studies showed the higher uptake of ^{99m}Tc-Digoxin in the heart of mice. Scintigraphic imaging indicated accumulation of ^{99m}Tc-Digoxin in heart of normal rabbits. These results endorse that ^{99m}Tc-Digoxin may be a selective imaging agent for myocardial imaging.

REFERENCES

Baggish AL, Charles MD and Boucher MD (2008). Radiopharmaceutical Agents for Myocardial Perfusion Imaging. *Circulation,* **118**: 1668-1674.

Banerjee J, Pillai MR and Ramamoorthy N (2001). Evolution of Tc-99m in diagnostic radio-pharmaceuticals. *Semin. Nucl. Med.,* **31**: 260-277.

Eberhard H, Gerald DR, David EK, Heinrich RS (1982). Tc-99m Dextran: A New Blood-Pool-Labeling Agent for Radionuclide Angiocardiography. *J. Nucl. Med.,* **23**: 348-353.

Debnath MC, Sarkar BR, Ganguly S and Banerjee S (2006). Preparation of ^{99m}Tc-labelled conjugates of ouabagenin and their biological evaluation in animal models. *Nucl. Med. Commun.,* **27**: 271-279.

Eisner DA and Smith TW (1991). *In*: Fozzard HA, Haber E, Katz AM, Morgan HE, eds. The Heart and Cardiovascular System. Raven, New York, p.863-902.

Fujibayashi Y, Takemura Y and Matumoto k (1992). High myocardial accumulation of radioiodinated digoxin derivative: A possible Na, K-ATPase imaging agent. *J. Nucl. Med.,* **33**: 545-549.

Fujibayashi Y, Takemura Y, Taniuchi H, Iijima N, Konishi J and Yokoyama A (1993). Basic evaluation of ^{67}Ga labeled digoxin derivative as a metal-labeled bifunctional radiopharmaceutical. *Ann. Nucl. Med.,* **7**:

239-244.

Gibbons RJ (2000). Myocardial perfusion imaging. *Heart*, **83**: 355-360.

Henzlova MJ, Cerqueira MD, Mahmarian JJ, Yao SS (2006). Stress protocols and tracers. *J. Nucl. Cardiol.,* **13**: 80-90.

Higley B, Smith F W, Smith T, Gemmell HG, Das Gupta P, Gvozdanovic DV, Graham D, Hinge D, Davidson J and Lahiri A (1993). Technetium-99m-1, 2- bis[bis(2-ethoxyethyl) phosphino]ethane: human biodistribution dosimetry and safety of a new myocardial perfusion imaging agent. *J. Nucl. Med.,* **34**: 30-38.

Ibrahim IT, Amin AM and El-Azony KM (2010) Preparation of Radioiodo-Metoprolol and Its Biological Evaluation as a Possible Cardiac Imaging Agent. *Radiochemistry,* **52**: 212-216.

Ibrahim T and Sanad MH (2013). Radiolabeling and Biological Evaluation of Losartan as a Possible Cardiac Imaging Agent. Radiochemistry., **55**: 336-340.

Jones AG (1995). Technetium in nuclear medicine. *Radiochim. Acta.,* **70**: 289-297.

Jurisson S, Berning D, Jia W and Ma D (1993). Coordination compounds in nuclear medicine. *M. Chem. Rev.,* **93**: 1137-1156.

Morrison AR and Sinusas AJ (2010). Advances in radionuclide molecular imaging of myocardial biology. *J. Nucl. Cardiol.,* **17**: 116-134.

Renata M, Piotr G (2012). Radiopharmaceuticals in cardiology. *Nucl. Med. Rev.,* **15**: 39-45.

Talaat HM and El-Mohty AA (2013). Preparation of 186Re-MIBI complex for myocardial perfusion imaging as potential replacement of analogues 99mTc-MIBIcomplex. *J. Radioanal. Nucl. Chem.,* **295**: 233-237.

Thomas HM and Markus S (2008). The Future of Cardiovascular Imaging in the Diagnosis and Management of Heart Failure, Part 1, Circ. Cardiovasc. Imaging, **1**: 58-69.

Udo H, Thomas JB and James M (2003). Use of New Imaging Techniques to Screen for Coronary Artery Disease. *Circulation,* **108**: 50-53.

Yang DJ, Kim CG, Schechter NR, Azhdarinia A, Yu D, Oh C, Bryant JL, Won J, Kim EE and Podoloff DA (2003). Imaging with ^{99m}Tc ECDG targeted at the multifunctional glucose transport system: Feasibility study with rodents. *Radiology,* **226**: 465-473.

Zeng H and Zhang H (2014). Synthesis and biological evaluation of fatty acids conjugates bearing cyclopentadienyl-donors incorporated[^{99m}Tc/Re(CO)3]$^{+}$ for myocardical imaging. *Eur. J. Med. Chem.,* **10**: 17.

Zhang J, Song Z, Feng C and Wang X (2009). Synthesis and biodistribution of a novel [^{99m}TcN(PNP5) (DMCHDTC)]$^{+}$ Complex as a potential myocardial perfusion imaging agent. *App. Radiat. Isot.,* **67**: 1661-1663.

Zhang J, Song Z and Wang X (2009). Synthesis of a bis(2,3-dimethylcyclohexyl-dithiocarbamato)-nitrido ^{99m}Tc complex: A potential tracer for myocardial imaging. *App. Radiat. Isot.,* **67**: 577-580.