DOI: 10.1007/s10967-007-7057-7 *Journal of Radioanalytical and Nuclear Chemistry, Vol. 277, No.2 (2008) 303–309*

Preparation, characterization and biodistribution of the ^{99m}Tc-salicylidenediamine-N-dione complex

M. Javed,[1] I. U. Khan,[1]* M. Naeem,[1] S. Asghar,[1] H. Naseer,[1] M. S. Iqbal,[2] J. Anwar[3]

[1] *Institute of Nuclear Medicine and Oncology (INMOL), Wahdat Road, P. O. Box 10068, Lahore-54600, Pakistan*
[2] *Department of Pharmacy, University of Sargodha, Pakistan*
[3] *Institute of Chemistry, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan*

(Received May 23, 2007)

A tetradentate set of N_2O_2 salicylaldehyde-amine-N-dione Schiff base was prepared by condensation with salicylaldehyde, ethylenediamine, 2,4-dione and reduction with $NaBH_4$. The ligand system was characterized by ^{1}H-NMR and FT-IR spectroscopy and HPLC. Radiolabeling studies of the ^{99m}Tc-complex were performed using stannous ions as the reducing agent. The purity of the complex was determined by ascending solvent system on paper chromatography and instant thin-layer chromatography (ITLC). The yield of the complex was >90%. Biodistribution of the ^{99m}Tc-complex of the precursor was studied in rabbits. A significant uptake and retention of injected activity was observed in the liver and cleared through the bladder. A faint activity was also observed in kidneys. These results indicate that the proposed system may be suitable for development of a liver/spleen imaging agent for future clinical applications.

Introduction

Earlier studies demonstrated that tetradentate amine-phenol ligands form neutral lipophilic technetium complexes in high yields.[1] These ^{99m}Tc-labeled amine phenols show outstanding characteristics in both chemical and biological areas concerning neutrality, lipophilicity, high ^{99m}Tc labeling yields, high leukocytes labeling yields and good in vitro stability.[1–5] In addition, transition metal[5] conjugated to Schiff's base ligands have also been widely investigated because of their preparative diversity, conformational variability and extensive usage in oxidative catalysis.[6–9] In the previous work, the amine-phenol ligands were synthesized by condensing one equivalent of triamines with two equivalents of salicylaldehyde and reducing the resultant Schiff's bases with $NaBH_4$. REFOSCO et al.[10] reported the synthesis and characterization of ^{99m}Tc complexes of the Schiff bases of similar pentadentate ligands.

The excellent complexation of the amine-phenol ligands with ^{99m}Tc and the high stability of the resultant complexes prompted us to study the feasibility of developing bifunctional ligands of the amine-phenol type for studying them as potential hepatobiliary agents. In this study, we have synthesized the Schiff's base by the reaction of salicylaldehyde with ethane 1,2-diamine and reduction with $NaBH_4$, followed by the condensation with pentane 2,4-dione. Afterwards, the complexation of the resultant NNOO tetradentate Schiff's base was performed with ^{99m}Tc and its biodistribution was carried out in rabbits and volunteers.

Experimental

Materials

Salicylaldehyde, ethane 1,2-diamine and molecular sieve 4 Å were purchased from Fluka, USA. Ethylacetate and methanol were purchased from Aldrich, USA. Acetonitrile, ethanol and CH_2Cl_2 were from Merck.

Equipment

Analytical high pressure liquid chromatography HPLC (Merck Hitachi), with interface D-7000, system controller, L-7200, pumps L-7100, diodes-array detector L-7455, degasser L-7612, HPLC-separating column C18 (Vydac) (250×4.6 mm^2; 5 μm; 300 A), was used in this study. EM 360 MHz nuclear magnetic resonance (NMR) spectrometer (Varian Int.) was used for identification of the compound and FT-IR spectra were recorded on Perkin-Elmer 882 spectrophotometer by using KBr disc technique.

Schiff base formation

0.1M of salicylaldehyde was dissolved in 80 ml acetonitrile (Step 1) and was added slowly during one hour to 0.6M of ethane 1,2-diamine. The resultant mixture was then stirred with a magnetic bar for half an hour, afterwards 10 g molecular sieve (4 Å) was added and stirring was continued overnight at room temperature. The solution was filtered and the solvent evaporated under vacuum by a rotary vaporator.

* E-mail: kiu786@hotmail.com

0236–5731/USD 20.00

Akadémiai Kiadó, Budapest
Springer, Dordrecht

A yellowish material was obtained, which was recrystallized from ethanol. Yellowish needle crystals were obtained and separated by filtration and dried: FT-IR gave (C=N) at 1633 cm^{-1} and –NH bond at 3435 cm^{-1}.

Reduction of azomethane bond

The residual material of the above reaction was suspended in 100 ml methanol in ice bath. 0.1M of $NaBH_4$ was slowly added by spatula in small portions within 30 minutes with constant magnetic stirring. After the completion of adding the reducing agent, the reaction mixture was stirred for 2 hours under the same conditions. The resultant mixture was further stirred for 30 minutes at room temperature. After this, the solvent was evaporated by rotary vaporator, followed by addition of 100 ml of distilled water and 0.2 g of NaOH. The aqueous phase was extracted with CH_2Cl_2 (3×50 ml). The combined organic phase was dried over anhydrous $MgSO_4$ overnight, filtered and the solvent evaporated by rotary vaporator.

Condensation with dione

10 mmol of crude product of the previous reaction was mixed slowly with 20 mmol pentane 2,4-dione in 10 ml CH_3CN and stirred with a magnetic bar for 3 hours at room temperature. The solvent was evaporated and a yellowish material was obtained. The reaction scheme is shown in Fig. 1.

Radiolabeling

10 mg ligand was dissolved in 10 ml ethanol with continuous stirring, followed by addition of 10 mg gentesic acid as an anti-oxidant (A). Afterwards, 50 mg of $SnCl_2{\cdot}2H_2O$ was dissolved in hot 0.5 ml conc. HCl and diluted in 10 ml 50% ethanol/water. 50 µl of $SnCl_2{\cdot}2H_2O$ solution was added to (A) and pH was adjusted to 8–8.5 with 1N NaOH and 0.1N NaOH. The resultant solution was dispensed in 1 ml volume into a 10-ml serum vial and 20 mCi $Na^{99m}TcO_4$ was added and incubated at room temperature for 15 minutes. The proposed structure after radiolabeling with ^{99m}Tc[11] is shown in Fig. 2.

Results and discussion

Analysis by FTIR

The FT-IR of the proposed tetradentate N_2O_2 ligand (Fig. 3) differs from the starting Schiff base notably in the absence of the band at 1634 cm^{-1}, assignable to the –C=N bond. The spectrum exhibited new FT-IR absorptions at 3284 cm^{-1} for –N–H bond and at 1598.5 cm^{-1} for the conjugation (C=C-C=O) bond based on the product of tetradentate salicylidenediamine-amino-N-dione structure.

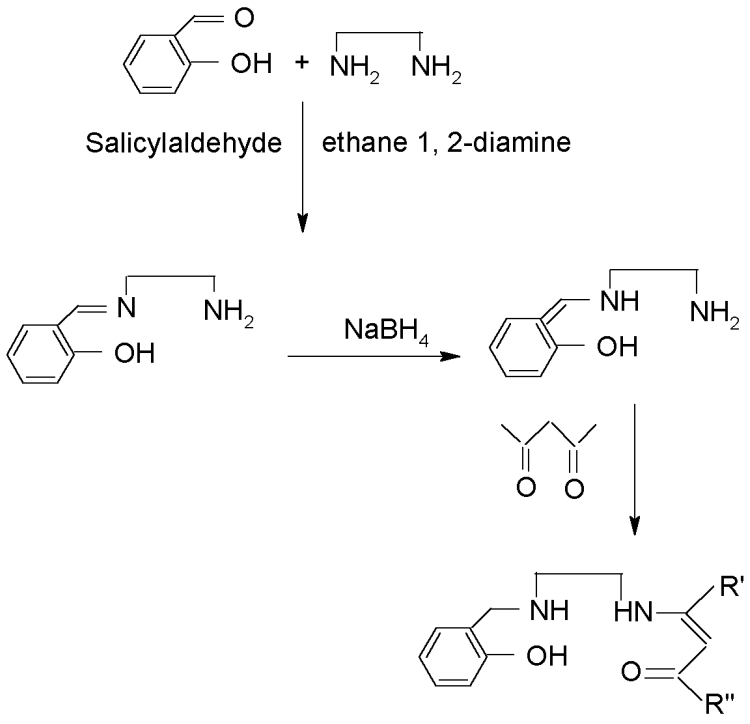

Fig. 1. Ligand synthesis of phenol-amine tetradentate. R' or R'' stands for methyl group

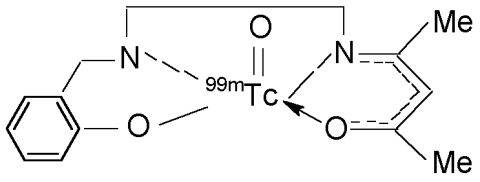

Fig. 2. Proposed structure after radiolabeling with ^{99m}Tc

Analysis by NMR

The salicylidenediamine-N-dione tetradentate set of the N_2O_2 ligand was also characterized by 60 MHz ^{1}H-NMR (Fig. 4). The spectra consisted of four distinct regions: methyl group (doublet), benzylic group (singlet), amine group (broad peak) and aromatic group (multiplet). Methyl proton resonance appeared as doublet in the region at 2.50–2.65 ppm, benzylic proton resonance appeared at 3.8 ppm as singlet and aromatic proton resonance appeared at 6.5–7.1 ppm as multiplet. Due to slow exchange of the secondary N–H bond, the amino proton was partly decoupled and a broad spectrum resulted at 6.5 ppm. The absorption due to the adjacent benzylic proton was not split and appeared at 3.1 ppm as singlet.

Analysis by HPLC

HPLC was performed by using a linear gradient of 0.08% TFA in acetonitrile (A) and 0.1% TFA in water (B) from 10 to 60% A in B over 30 minutes at a flow rate of 0.6 ml/min. The retention time of the product was found at 23.4 minutes. The chromatogram is shown in Fig. 5.

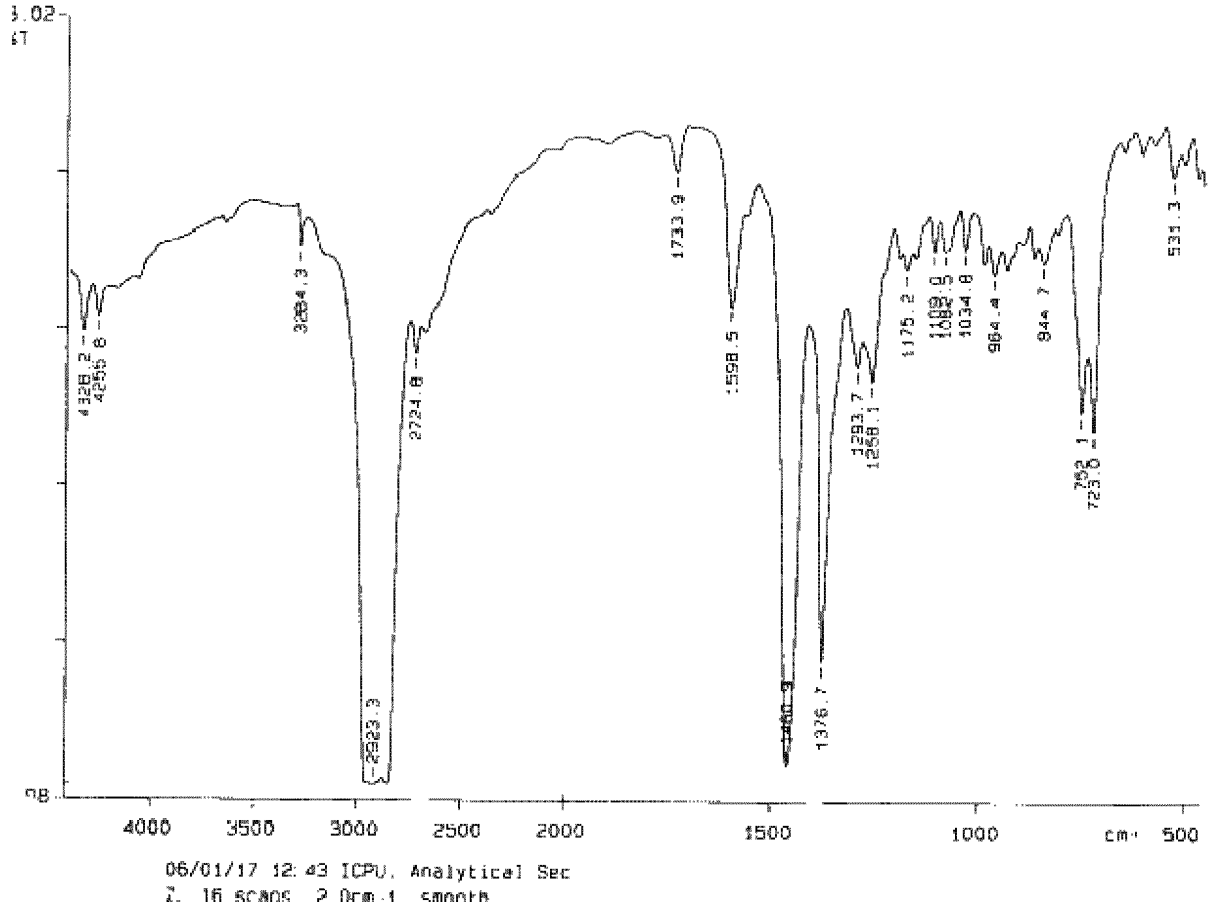

Fig. 3. FTIR of Schiff's base of the product

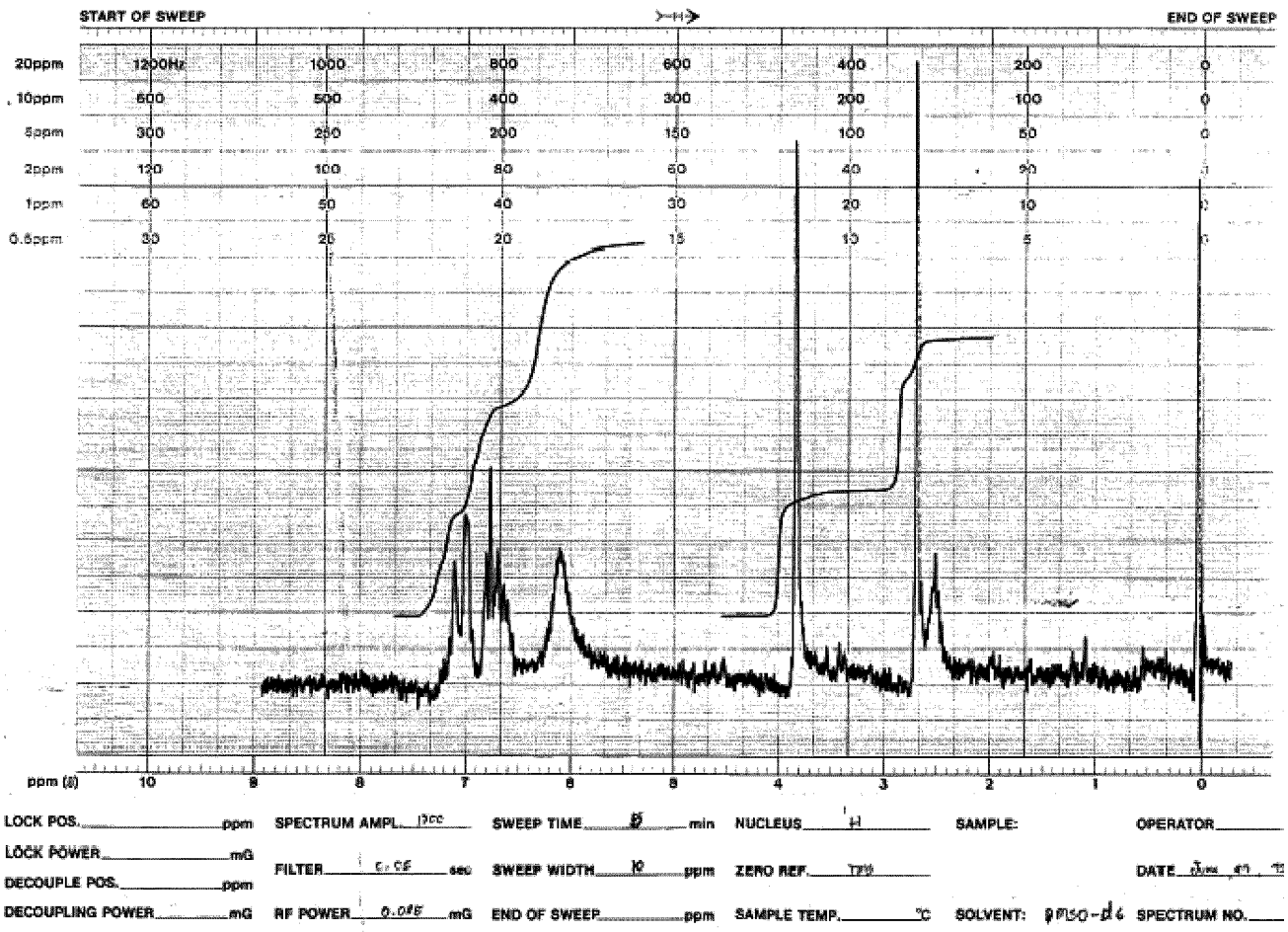

Fig. 4. NMR of proposed salicylidenediamine-N-dione complex

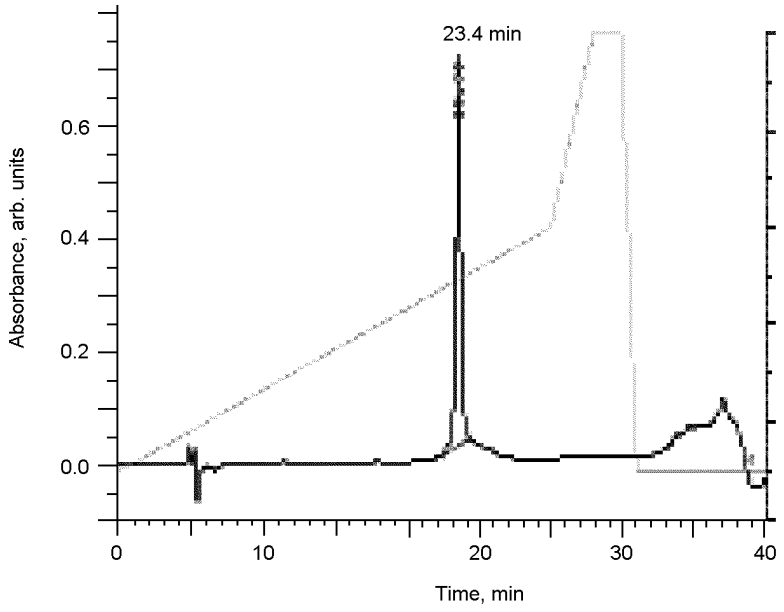

Fig. 5. HPLC chromatogram of compound developed by 10% to 60% ACN in 30 minutes

Quality control of the labeled compound

Radiochemical purity was studied in acetone and saline. More than 95% of reduced ^{99m}Tc was bound to the ligand. Three species were formed during labeling of the proposed phenol-amine-one ligand with ^{99m}Tc eluted from a ^{99}Mo–^{99m}Tc generator. The bound ^{99m}Tc-complex, impurities such as ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) and reduced hydrolyzed $^{99m}TcO_2$, were determined by ITLC, Whatmann No. 3 MM chromatography paper with acetone as mobile phase. In this system, $^{99m}TcO_4^-$ and ^{99m}Tc-complex have an R_f of 0.8–0.9, while the reduced hydrolyzed $^{99m}TcO_2$ appears at R_f=0.00–0.01. Free $^{99m}TcO_4^-$ was separated in saline, where the ^{99m}Tc-complex and $^{99m}TcO_2$ remained at the origin, while $^{99m}TcO_4^-$ appeared at R_f=0.9–1.0. Labeling yield of ^{99m}Tc-complex was more than 95%.

Biodistribution in rabbits and patients

The biodistribution of the proposed ^{99m}Tc labeled ligand was determined in rabbit. Data are shown in Fig. 6. During one hour dynamic study of rabbit injected with 2 mCi of the ^{99m}Tc-complex through ear vein, radioactivity was accumulated in the liver, however, no excretion of the tracer was noted by the gall bladder. Excretion of the tracer was noted from both kidneys to urinary bladder. This is in accordance with findings described by HUANG et al.[12]

Delayed static views of brain shows no tracer uptake in brain (Fig. 7). Similarly, no tracer uptake is noted in the myocardium. Marked tracer uptake is noted in the liver, and the spleen is faintly visualized.

Due to the high uptake of ^{99m}Tc-salicylidenediamine-N-dione in the liver, it was further evaluated in a healthy volunteer. Imaging of volunteer showed a gradual and adequate tracer clearance from the blood by the liver. Adequate homogenous tracer uptake is noted in the liver; however, delayed images till 30-minute p.i. did not show clearance from the gall bladder. This indicates that the tracer is not excreted through the hepatobiliary route. Both kidneys were faintly visualized and activity was collecting in the urinary bladder. This confirms that ^{99m}Tc-salicylidenediamine-N-dione complex is excreted through the urinary system (Fig. 8).

No uptake in the brain and myocardium were found, which shows that the ^{99m}Tc-salicylidenediamine-N-dione ligand is not lipophilic and does not cross the blood brain barrier. The thyroid gland is not visualized (Fig. 9).

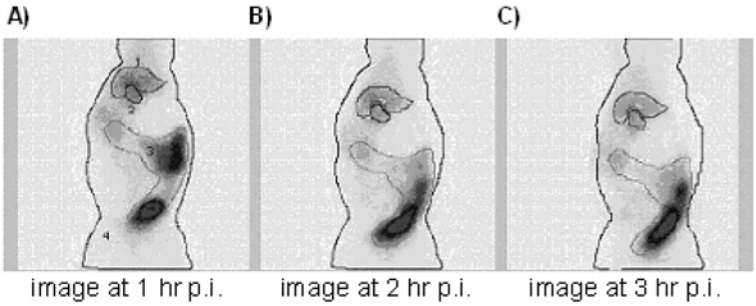

Fig. 6. Biodistribution of ^{99m}Tc-complex in a rabbit

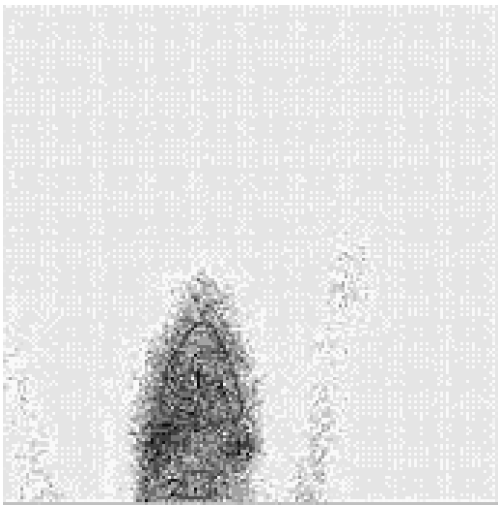

Fig. 7. Profile showing almost background activity in rabbit brain

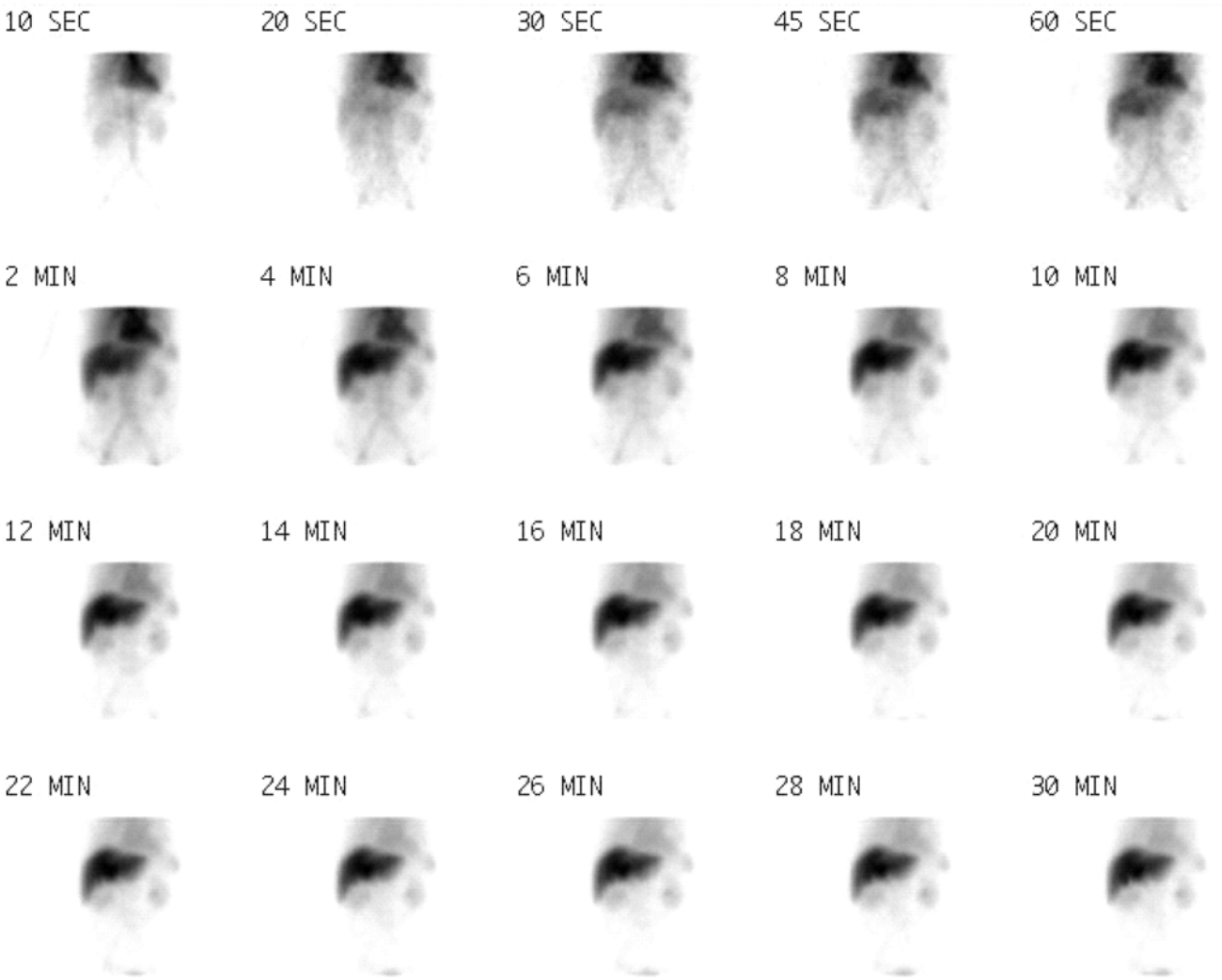

Fig. 8. Profile showing excretion of ^{99m}Tc-complex from blood pool in a normal volunteer in a dynamic flow till 30 minutes

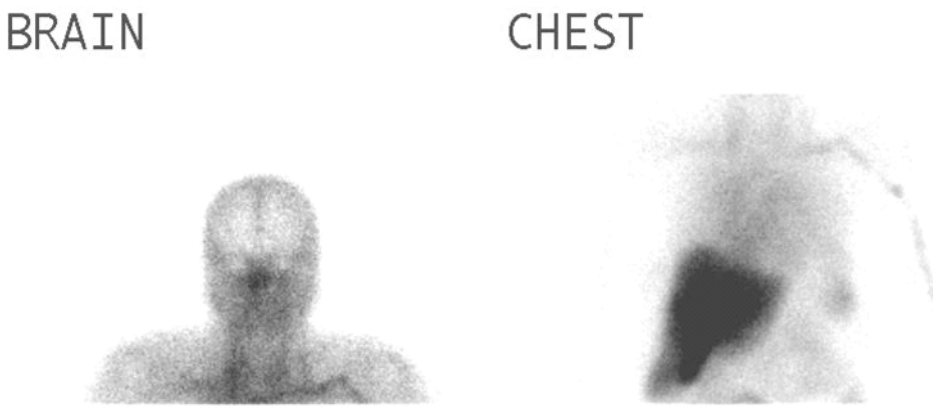

Fig. 9. Static image of brain and myocardium of healthy volunteer showing poor tracer uptake by brain and myocardium

High liver uptake suggests metabolism in the liver. However, there is no evidence of hepatobiliary excretion of tracer. Uptake in spleen is most probably due to blood pooling of radiotracer.

Conclusions

The incidence of hepatocellular carcinoma is gradually increasing in Pakistan. Thousands of people in Pakistan die every year due to this problem. So the high uptake of proposed ^{99m}Tc-complex of tetradentate ligand in liver encourages further studies with hepatoma bearing models.

References

1. M. R. A. PILLAI, C. S. JOHN, J. M. LO, D. E. TROUTNER, Appl. Radiation Isotopes, 41 (1990) 557.
2. M. CORLIJA, W. A. VOLKERT, Intern. J. Radiat. Appl. Instr., B18 (1991) 167.
3. M. R. A. PILLAI, K. KOTHARI, E. O. SCHLEMPER, M. CORLIJA, R. A. HOLMES, W. A. VOLKERT, Appl. Radiation Isotopes, 44 (1993) 1113.
4. J. M. LO, K. S. LIN, C. Y. HUANG, Appl. Radiation Isotopes, 45 (1994) 587.
5. M. R. A. PILLAI, C. S. JOHN, J. M. LO, E. O. SCHLEMPER, D. E. TROUTNER, Inorg. Chem., 29 (1990) 1850.
6. R. D. ARCHER, B. WANG, Inorg. Chem., 29 (1990) 39.
7. K. HAMACHI, R. IRIE, T. KATSUKI, Tetrahedron Lett., 37 (1996) 4979.
8. C. H. SENANAYAKE, G. B. SMITH, K. M. RYAN, Tetrahedron Lett., 37 (1996) 3271.
9. Y. S. SHARMA, H. N. PANDEY, P. MATHUR, Polyhedron, 13 (1994) 3111.
10. R. FIORENZO, T. FRANCESCO, M. ULDERIO, J. Chem. Soc. Dalton Trans., (1988) 611.
11. G. F. MORGAN, M. DEBLATON, P. CLEMENS, P. V. DEN BROECK, A. BOSSUYT, J. Nucl. Med., 32 (1991) 500.
12. W. HUANG, H. YAO, J. LO, Ann. Nucl. Med. Sci., 17 (2004) 13.