

THE REEVALUATION OF MIBI AS THE LIGAND OF ^{99m}Tc -MIBI RADIOPHARMACEUTICAL FOR MYOCARDIAL IMAGING

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ABSTRACT

THE REEVALUATION OF MIBI AS THE LIGAND OF ^{99m}Tc -MIBI RADIOPHARMACEUTICAL FOR MYOCARDIAL IMAGING. Methoxy isobutyl isonitrile labelled with technetium-99m (^{99m}Tc) radionuclide can be used for the evaluation of acute myocardial infarction. The ^{99m}Tc -MIBI radiopharmaceutical is available in the lyophilized-kit, which is separately packed with its radionuclide. Recently, in CNTMR-BATAN Bandung, ^{99m}Tc -MIBI radiopharmaceutical was prepared using MIBI ligand synthesized in 1996 as lyophilized-kits having the radiochemical purity less than 90 % and not accumulated in the myocardium. It could be caused by several factors, among others were technical preparation of lyophilized dry-kit and the quality of MIBI; therefore the reevaluation of the ligand for the quality improvement was needed. Firstly, MIBI ligand was recrystallized. It was found that MIBI as $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt was a glassy solid with the melting point of 98 -99 °C. The infra red analysis showed the presence of spectra vibration at 1085, 1180 cm^{-1} and 2180, 2220 cm^{-1} for $-\text{OCH}_3$ and $\text{N}\equiv\text{C}$ groups, respectively. The preparation of ^{99m}Tc -MIBI was performed by adding ^{99m}Tc radionuclide into new MIBI liquid-kit, then boiled in water bath for minimum period of 10 minutes. The radiochemical purity of ^{99m}Tc -MIBI was analysed by thin layer chromatographic method using absolute ethanol as a mobile phase. The pre-clinical evaluation was observed in Wistar rat and clinical studies were performed in volunteer using gamma camera. It is revealed that the radiochemical purity of more than 90 % i.e. 96.83 ± 1.43 % was still stable until 24 hours storage at room temperature. The stability studies of ^{99m}Tc -MIBI in human blood plasma showed that in 6 hours, the radiochemical purity was decreased to 90.89 ± 2.90 %. Clinical application of ^{99m}Tc -MIBI with tomography techniques using gamma camera showed the distinct accumulation of radioactivity in the heart.

Key words : Radiopharmaceutical, MIBI, ^{99m}Tc , Myocardial imaging.

INTRODUCTION

Over the years, thallium-201 (^{201}Tl) has been the main radionuclide for the assesment of myocardial perfusion and the diagnosis of coronary artery diseases. However, due to several disadvantages such as unsatisfactory nuclide properties, limited availability, high cost and biological redistribution in human body, the replacement of ^{201}Tl by an agent based on a more reliable isotop has been a long standing goal in nuclear medicine. Recently, several ^{99m}Tc -cationic isonitrile compounds have been developed to dissolve these problems. Technetium (^{99m}Tc) is chosen because cheap and emitted gamma photon of 140 keV that suitable for gamma camera imaging [1].

^{99m}Tc -methoxy isobutyl isonitrile (^{99m}Tc -MIBI) is one of promising ^{99m}Tc -labeled radiopharmaceutical as myocardial agent [2]. Myocardial

perfusion imaging using this radiopharmaceutical can evaluate the ischemic condition, due to oxygen demand greater than the local oxygen supply in the myocardium especially in patient with acute myocardial infarction. On the other hand, ^{99m}Tc -MIBI radiopharmaceutical can be used for studying non cardiac tissues such as mammary tumours [3], parathyroid and whole body scan [4].

Previous work, MIBI in the form of $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt has been synthesized successfully in laboratory scale at CNTMR-BATAN Bandung [5]. The investigation was continued with development of formulation of ^{99m}Tc -MIBI [6]. In the clinical application, ^{99m}Tc -MIBI radiopharmaceutical was utilized in MIBI lyophilized-kit, which is separately packed with its radionuclide. The MIBI lyophilized-kit was formulated using an optimal condition obtained in previous investigation and ^{99m}Tc radionuclide was added immediately to lyophilized-kit before used, according to the MIBI labelling protocol [6].

Until now, the preparation of MIBI lyophilized-kit still use $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt synthesized in 1996 at CNTMR-BATAN Bandung. The radiochemical purity of ^{99m}Tc -MIBI radiopharmaceutical prepared using the MIBI lyophilized-kit was less than 90 %. Meanwhile according to Dupont, radiochemical purity requirement for ^{99m}Tc -MIBI radiopharmaceutical has to be more than 90 % [7]. The decrease of radiochemical purity of CNTMR-BATAN Bandung ^{99m}Tc -MIBI radiopharmaceutical could be caused by several factors, such as lyophilized-kit preparation process including cooling, drying, sealing of vial for vacuum condition and also type of vial and septa used. The other important factor was the stability of $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt ligand, due to it was synthesized in 1996.

The purpose of this study is to confirm the stability of $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt by evaluating its physical-chemical characteristic, the preparation of MIBI liquid-kit followed by labelling with ^{99m}Tc radionuclide. The stability of ^{99m}Tc -MIBI labeled compound was evaluated during storage in room temperature and *in-vitro* stability test in the human blood plasma at 37°C. Pre-clinical study was done in Wistar rat, while the clinical evaluation of ^{99m}Tc -MIBI was conducted to the volunteer

EXPERIMENTAL METHODS

The materials used were MIBI in the form of $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt synthesized by CNTMR-BATAN Bandung, sodium pertechnetate solution from ^{99}Mo - ^{99m}Tc generator (PT Batan Technology). L-cysteine hydrochloride monohydrate (Sigma), sodium citrate, mannitol, ethanol, TLC- Al_2O_3 plate (E.Merck), bacteria filter (0.22 μm , Millipore) and animal Wistar rat (body weight 160-200 g).

The equipments used were infra red spectrometer, UV spectrometer, melting block apparatus, singlechannel analyzer, dose calibrator, thin layer chromatography apparatus, animal scanner and gamma camera.

Analysis of MIBI

Analysis of MIBI was carried out by infra red spectrometer, UV spectrometer, melting point apparatus as well as by visual method, including the form, colour and odor of $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt.

Labelling with $^{99\text{m}}\text{Tc}$ radionuclide

Labelling of MIBI with $^{99\text{m}}\text{Tc}$ radionuclide was directly performed using liquid-kit prepared the same composition as that MIBI lyophilized-kit. Five mg of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ was dissolved in 0.1 mL of 1N HCl, the final volume was adjusted to 5 mL with sterile water for injection (SWFI), and the solution was then labelled as solution A. Five mg of $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt was added into 8 mL of SWFI and stirred until it was completely dissolved. Then 10 mg of L-cysteine hydrochloride monohydrate, 26 mg of sodium citrate and 200 mg of mannitol were added, and stirred respectively until dissolution and then 0.6 mL of solution A was added. The final volume was adjusted to pH 5.5 – 6.0 with 0.1N NaOH/0.1N HCl. Final volume was adjusted to 10 mL with SWFI. One mL of this solution was dispensed in a 10 mL borosilicate vial and added 1.0 – 2.0 mL (5 – 10 mCi) of $^{99\text{m}}\text{Tc}$ generator eluate. The vial was swirled for a few seconds and placed in a boiling water bath for minimum period of 10 minutes.

Determination of Radiochemical Purity of $^{99\text{m}}\text{Tc}$ -MIBI

Radiochemical purity of $^{99\text{m}}\text{Tc}$ -MIBI was determined using thin layer chromatography method. Two drops of $^{99\text{m}}\text{Tc}$ -MIBI solution were applied side by side on top of a drop of pre-applied absolute ethanol wet spot on TLC- Al_2O_3 strip (1x10 cm). The TLC-strip was developed with absolute ethanol in chromatographic chamber. Each cm of TLC-strip was cut and counted in gamma scintillation counter.

Stability Test of $^{99\text{m}}\text{Tc}$ -MIBI

After labelling MIBI liquid-kit with $^{99\text{m}}\text{Tc}$ radionuclide, the solution was kept in the lead pot at room temperature. The radiochemical purity of $^{99\text{m}}\text{Tc}$ -MIBI was checked at 0, 6 and 24 hours by TLC- Al_2O_3 strip according to the procedures described earlier.

Stability Test of ^{99m}Tc -MIBI in Human Blood Plasma *in-vitro*

The *in-vitro* stability of ^{99m}Tc -MIBI in human blood plasma was determined at 37 °C as described by Steffens [8]. Two hundred and fifty μL of ^{99m}Tc -MIBI was added into 500 μL of human blood plasma and stirred vigorously. Furthermore it was kept in lead pot at 37 °C then the plasma sample was analyzed by TLC- Al_2O_3 strip at 0, 2, 4, 6 and 24 hours incubation.

Pre-clinical Evaluation of ^{99m}Tc -MIBI

The pre-clinical study was conducted in Wistar rat. After labelling MIBI liquid-kit with ^{99m}Tc radionuclide, 200 μCi in 100-200 μL of ^{99m}Tc -MIBI was injected into the rat tail vein. Biodistribution of ^{99m}Tc -MIBI was determined by animal scanner at 30 minutes after injection.

Clinical Application of ^{99m}Tc -MIBI

Clinical application of ^{99m}Tc -MIBI was performed at Nuclear Medicine Department, Hasan Sadikin Hospital. One mL of MIBI liquid-kit was filtered through 0.22 μm Millipore filter into a steril vial and 1-2 mL (20-30 mCi) of ^{99m}Tc from ^{99}Mo - ^{99m}Tc generator was added. The vial was swirled for a few seconds and placed in a boiling water bath for minimum period of 10 minutes and was stored at room temperature. One mL (10 – 15 mCi) of ^{99m}Tc -MIBI was injected intravenously to the volunteer at stress condition (after exercise). Imaging was performed on gamma camera 1 hour after injection.

RESULTS AND DISCUSSION

Methoxy isobutyl isonitrile is the unstable compound in the liquid form with very strong odor and toxic. Because of that, MIBI has been prepared in the stable form of tetra (2-methoxy isobutyl isonitrile) Copper (I) tetra fluoroborate, $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt, odorless, non toxic which is suitable for lyophilized-kit MIBI formulation.

According to $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt has been synthesized at BATAN-Bandung in 1996, therefore the various reevaluation on the stability of the compound including physical-chemical analysis and visual evaluation as well, should be carried out. The visual testing results showed that MIBI as $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt still have a glassy solid form, odorless with the melting point of 98 - 99 °C and 99 – 100 °C for MIBI synthesized in 1996 and re-crystallized in 2006, respectively. This melting point is different with melting point obtained beforehand [5].

$[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt was characterized by infra red spectrometer. It was found the same spectrum absorption as the previous characterization for the specific groups [5, 9], those were at 2220, 2180 cm^{-1} for $\text{N}\equiv\text{C}$ group,

as well as, at 1180, 1085 cm^{-1} for methoxy group ($-\text{OCH}_3$). The infra-red spectrum of $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt was showed in Figure 1. The characterization test of $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt using ultra violet spectrometer showed that both of sample had the maximum absorption at 249 nm (Figure 2).

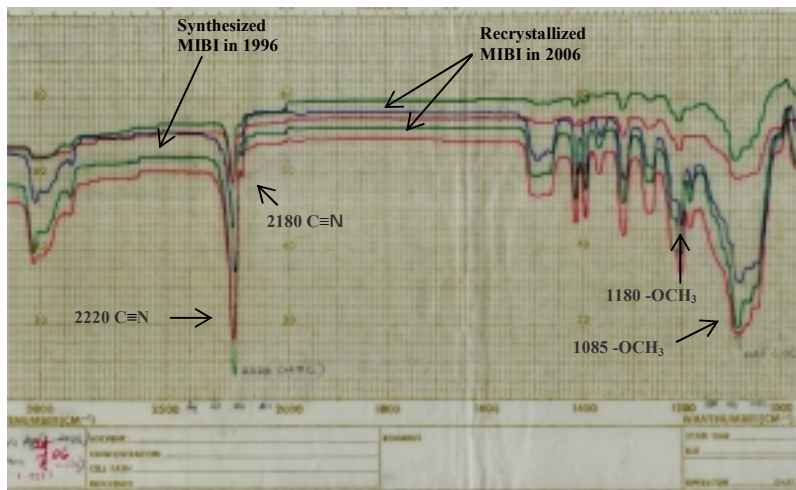


Figure 1. Infra-red spectrum of $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt, synthesized in CNTMR-BATAN Bandung.

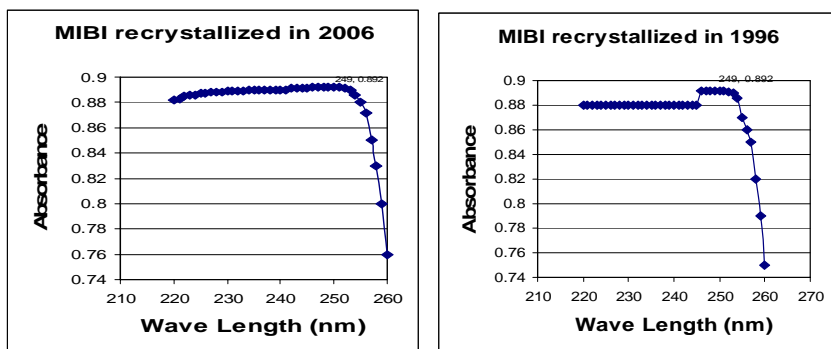


Figure 2. UV spectrum of $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt, synthesized in CNTMR-BATAN Bandung.

The decrease of radiochemical purity of ^{99m}Tc -MIBI prepared using lyophilized-kit could be caused by various factors, such as the stability of $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt as a ligand. In order to prove whether the $\text{Cu}(\text{MIBI})_4\text{BF}_4$ salt still could be used or not as a ligand for the preparation of ^{99m}Tc -MIBI radiopharmaceutical, the labelling with ^{99m}Tc radionuclide was performed by using the MIBI liquid-kit. The labelling efficiency was expressed as the radiochemical purity of ^{99m}Tc -MIBI that was determined by thin layer chromatography. In this system, ^{99m}Tc -MIBI migrated with the front of the mobile phase ($R_f = 0.9 - 1.0$), and the impurity in the form of $(^{99m}\text{TcO}_4)^-$ migrated with $R_f = 0.25 - 0.5$, meanwhile the impurity of ^{99m}Tc colloid ($^{99m}\text{TcO}_2$) was found at the origin of the strip ($R_f = 0.00$). Using this system, the both labelled compounds having the radiochemical purity more than 90 %, those were 95.78 ± 1.08 % and 95.07 ± 1.57 % for MIBI synthesized in 1996 and recrystallized in 2006, respectively. Based on these results it could be explained that both $\text{Cu}(\text{MIBI})_4\text{BF}_4$ salt still fulfill the requirements for ligand in the preparation of ^{99m}Tc -MIBI radiopharmaceuticals. Moreover, it was seen that both ^{99m}Tc -MIBI radiopharmaceuticals gave the almost same radiochemical purity. The results ($n = 11$) was shown in Figure 3.

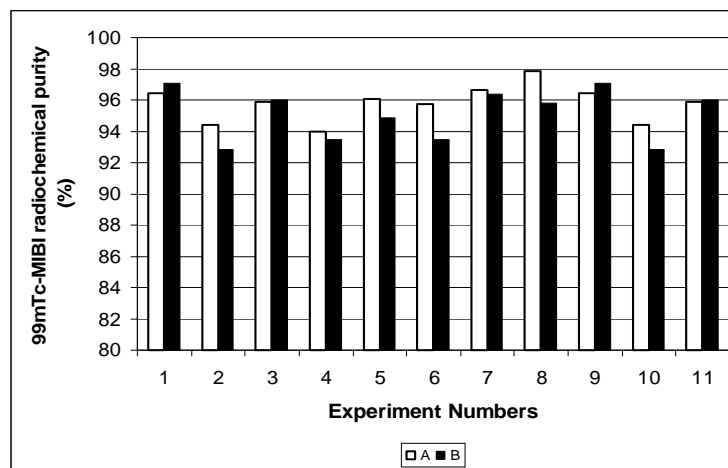


Figure 3. Radiochemical purity of ^{99m}Tc -MIBI prepared using MIBI liquid-kit. A : MIBI synthesized in 1996 (◊-◊); B : MIBI recrystallized in 2006 (■-■).

From several previous results, it had been reported that the radiochemical purity of ^{99m}Tc -MIBI prepared using MIBI lyophilized-kit were less than 90 %, thus were not fulfill the requirement of the

radiopharmaceutical quality. However ^{99m}Tc -MIBI that was formulated using MIBI liquid-kit gave more than 90 % of radiochemical purity. Therefore, the decrease of chemical purity of ^{99m}Tc -MIBI prepared by using MIBI lyophilized-kit was suspected caused by various factors in the lyophilization process, i.e. vacuum condition, refrigeration and drainage. The inappropriate couple of vial and septa resulted the vacuum condition was difficult to achieved, that could influence the stability of reductor used.

^{99m}Tc -MIBI could be used as the multidose radiopharmaceuticals, therefore, it is important to know the stability during storage at room temperature. In this study, ^{99m}Tc -MIBI radiopharmaceutical was stable for at least 6 hours after preparation with the average radiochemical purity of 95.57 ± 1.96 %. While, 24 hours after preparation, the radiochemical purity was still more than 90 %. The results (n = 5) was shown in Figure 4.

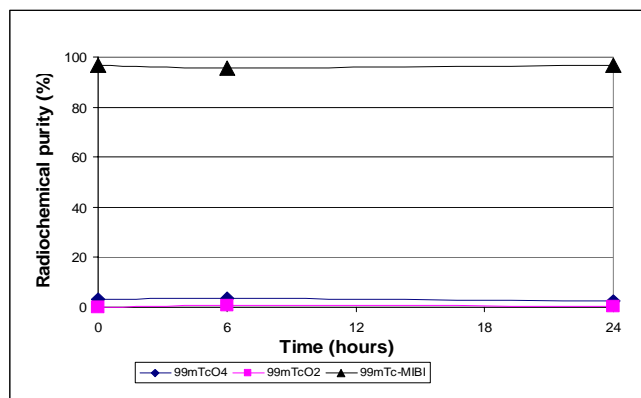


Figure 4. Stability of ^{99m}Tc -MIBI prepared using MIBI liquid-kit at room temperature.

The successful of the clinical use of radiopharmaceutical was also based on its stability in blood plasma. In order to know the stability of ^{99m}Tc -MIBI radiopharmaceutical in human body, the *in-vitro* determination of the radiochemical purity in human blood plasma was done at 37°C . ^{99m}Tc -MIBI was moderately stable with slow release of labelled compound during incubation in blood plasma. It was found that during 6 hours incubation at 37°C , ^{99m}Tc -MIBI has 90.80 ± 2.90 % of radiochemical purity and 88.53 ± 3.07 % of radiochemical purity was observed after 24 hours incubation. The results of ^{99m}Tc -MIBI stability in human blood plasma *in-vitro* could be seen in Figure 5.

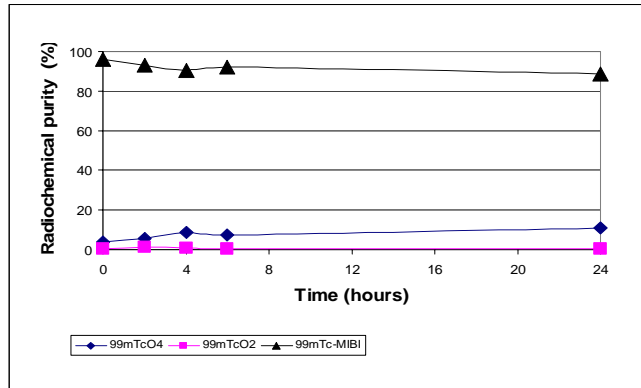


Figure 5. Stability *in-vitro* of ^{99m}Tc-MIBI prepared using MIBI liquid-kit in human blood plasma at 37 °C.

Although ^{99m}Tc-MIBI fulfill the requirement of the radiopharmaceutical, the suitability for the purpose in nuclear cardiology must be tested. Therefore, in order to know as well as to prove this case, it must be supported by biological test. The accumulation of radiopharmaceutical into the organ could be monitored with pre-clinical studies in animal using animal scanner. The scintigraphic images of ^{99m}Tc-MIBI into organs of Wistar rat 1 hour after injection was shown in Figure 6.

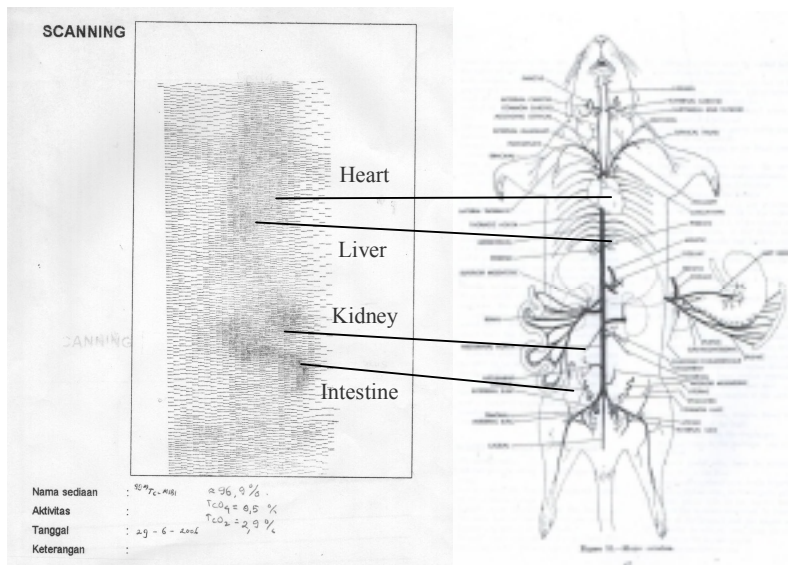


Figure 6. Biodistribution of ^{99m}Tc-MIBI 30 minutes after injection in Wistar rat using animal scanner.

The result indicated that ^{99m}Tc -MIBI was accumulated in several organs, including the heart, liver, kidney and intestines. Topographically, the heart is adjacent to the liver and the lungs, so it was rather difficult to visualize the results of scintigraphy method using animal scanner. However, generally radioactivity in the liver will descend quickly compared with radioactivity accumulated in the heart [10].

Moreover, the clinical evaluation of ^{99m}Tc -MIBI radiopharmaceutical to the normal volunteer using gamma camera has been carried out in Hasan Sadikin hospital. Figure 7 showed the heart images of ^{99m}Tc -MIBI radiopharmaceutical in normal volunteer. It was found that ^{99m}Tc -MIBI radiopharmaceutical was accumulated in the heart with satisfactory results. Therefore, this indicates that ^{99m}Tc -MIBI could be used clinically for myocardial imaging with good quality visualization.

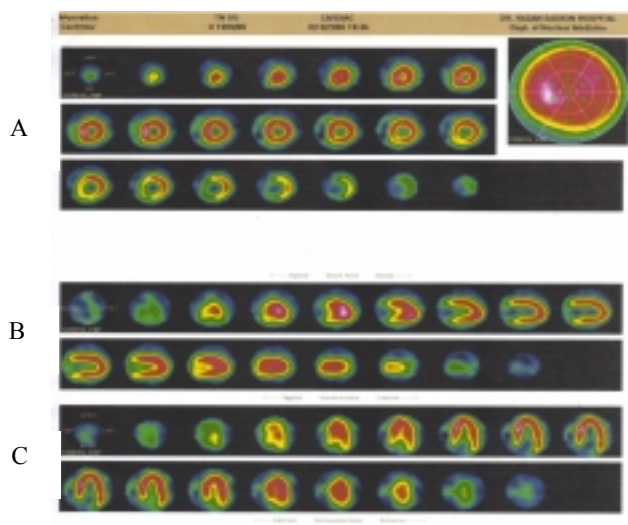


Figure 7. Heart imaging of ^{99m}Tc -MIBI one hour after injection in normal volunteer using gamma camera. A : frontal; B : sagittal; C : transversal.

CONCLUSION

The result of this study indicated that MIBI in the form of $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ salt, both compounds, synthesized in 1996 and recrystallized in 2006 still could be used as a ligand for the preparation of ^{99m}Tc -MIBI radiopharmaceutical. However, special precaution should be taken during the kit preparation process in order to achieve satisfactory labelling ($\geq 90\%$) for further clinical application.

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