THE EVALUATION OF SENSITIVITY AND SPECIFICITY OF NEW DEVELOPED 99M Tc- OFLOXACIN RADIOTRACER TO DISCRIMINATE INFECTION INDUCED BY STAPHYLOCOCCUS AUREUS AND STERILE INFLAMMATION IND…

Article *in* International Journal of Pharmaceutical Sciences and Research · January 2017

CITATION
1

READS
40

1 author:

Alireza Doroudi
Ahvaz Jundishapur University of Medical Sciences
32 PUBLICATIONS **132** CITATIONS

SEE PROFILE

Annals of Nuclear Medicine

Clinical Application of Ultrasound for Preparation of 99mTc-Sestamibi Complex

--Manuscript Draft--

Manuscript Number:	ANME-D-14-00246R3
Full Title:	Clinical Application of Ultrasound for Preparation of 99mTc-Sestamibi Complex
Article Type:	Original article
Corresponding Author:	Alireza Doroudi, Ph.D School of pharmacy of Ahvaz Jundishapur University of Medical Sciences Ahvaz ,Iran Ahvaz, Khuzestan IRAN, ISLAMIC REPUBLIC OF
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	School of pharmacy of Ahvaz Jundishapur University of Medical Sciences Ahvaz ,Iran
Corresponding Author's Secondary Institution:	
First Author:	Alireza Doroudi, Ph.D
First Author Secondary Information:	
Order of Authors:	Alireza Doroudi, Ph.D
	Mostafa Erfani, Assistant professor
	Behzad Norouzi, Pharm-D Student
	Seyyed Mostafa Saadati, Assistant professor
	Ali Kiasat, Assistant professor
	Faramarz Ahmadi, Assistant professor
	Behrooz Etesami
	Mohammad Hadi Baghersad
Order of Authors Secondary Information:	
Abstract:	Objective The main aim of this investigation is the clinical application of Ultrasound irradiation technique as an alternative method to reconstitute sestamibi kits in comparison of water boiling bath method. Methods The740-3700MBq(20-100 mCi)99mTc-MIBI (Sestamibi) complex samples were prepared due to ultrasound irradiation technique or boiled water bath method as a standard method. Twenty patients (8 men and 12 women; age range 30-72, median 52.45years) have been referred to Golestan hospital (Ahvaz, Khuzestan, Iran) for myocardial perfusion imaging (MPI). The subjects have been divided randomly into group A (3 men, 7 women, age rang 36-67, median 51.7 years) and group B (5 men, 5 women, age range 30-72, median 50.3 years) respectively. The 99mTc-MIBI radiopharmaceuticals have been prepared by Ultrasound irradiation technique administrated to group A and 99mTc-MIBI complex samples due to the boiled water bath technique administrated to the other group. For all patients the protocol two day stress/rest MPI was performed. Results The radio-HPLC and TLC studies have indicated that the 99mTc-MIBI complex samples with good yields could be prepared successfully due to new developed technique. The scintigraphy imaging studies have demonstrated the99mTc-Sestamibi prepared due to above mention modalities very identical biodistribution in the heart, thyroid, lung, liver, gallbladder, kidneys, stomach, large intestine and bladder of the subjects. Any unexpected accumulation of radiotracer samples have not observed in our approach. Conclusions The ultrasound irradiation technique is convenient and sufficient method to prepare

	99mTc-Sestamibi .It can be recommended as an alternative method to reconstitute Sestamibi kits particularly in emergency situations in order to reduce potentially medical risk by avoiding any delay in acute therapy for myocardial infarction. Key words: Sestamibi, 99mTc , 99mTc-MIBI, Ultrasound Irradiation
Author Comments:	Dear Seigo Kinuya Thank you very much for your kindness and provided me the other opportunity to submit my research article .I was trying to correct the manuscript according to the journal format. Any hints would be appreciated in order to increase the chance of this paper for publication in annals nuclear medicine. Best Regards
Response to Reviewers:	I have responded specifically to each comment. To make the changes easier to identify where necessary, I have numbered them. 1-Page 1: heat changed to heart 2-Page 3,10, and 11 : 99m pertechnetate changed to 99mTc pertechnetate 3-Page 3,4,7,15 and 16 : Bq symbol changed to MBq 4-Page 11: Line 3 from the top was corrected.

Dear Dr Seigo Kinuya

Editor-in-chief of Journal Annals of Nuclear Medicine

Thank you for giving me the opportunity to revise and resubmit this manuscript again. I appreciate the time and details provided by each honor reviewer. I have incorporated the suggested comments into the manuscript to the best of my ability. The manuscript has certainly benefited from these insightful revision suggestions. I look forward to working with you and the reviewers to move this manuscript closer to publication in the journal Annals Nuclear Medicine. I have responded specifically to each comment. To make the changes easier to identify where necessary, I have numbered them.

1- Page 1: heat changed to heart
2- Page 3,10, and 11 : 99m pertechnetate changed to 99mTc pertechnetate
3- Page 3,4,7,15 and 16 : Bq symbol changed to MBq
4- Page 11: Line 3 from the top was corrected.

As you notice, we agree with all the comments raised by the reviewers. We would like to take this opportunity to express our sincere thanks to the reviewers who identified area of our manuscript that needed corrections.

I hope the corrections which are presented on the basis of referee's comments would justify the revised manuscript for publication and this manuscript will be recognized suitable for publication in Annals Nuclear Medicine Journal.

Best Regards

Alireza Doroudi

Assistant professor of Radiopharmacy

Department of Medicinal Chemistry

School of Pharmacy, Ahvaz Jundishapur University of medical Sciences

Title Page w/ ALL Author Contact Info.

Alireza Doroudi [1*], Mostafa Erfani [2], Behzad Norouzi [1], Seyyed Mostafa Saadati [3], Ali Kiasat[3], Faramarz Ahmadi[3], Behrooz Etesami[3] ,Mohammad Hadi Baghersad[4]

Clinical Application of Ultrasound for Preparation of ^{99m}Tc-Sestamibi Complex

Short title: Sestamibi and Ultrasound irradiation

[1]School of Pharmacy ,Ahvaz Jundishapur University of Medical Sciences Ahvaz, Iran. [2]

[2]Radiation Application Research School, Nuclear Science and Technology Research Institute, [NSTRI], Tehran, Iran

[3] Nuclear Medicine Department – Golestan general hospital, Ahvaz Jundishapur University of Medical Sciences. Ahvaz Iran

[4]Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran ,Iran

* Corresponding author .Tel:986133738378, Fax: 986133738381

E-Mail address: Doroudi-a@ajums.ac.ir

Dr Seyyed Mostafa Saadati: E-Mail Address;Saadati_mostafa@yahoo.com

Nuclear Medicine Department – Golestan general hospital, Ahvaz Jundishapur University of Medical Sciences. Ahvaz Iran Tel: 986133743057

Dr Faramarz Ahmadi : E-mail address;Faramarz.ahmadi@gmail.com

Nuclear Medicine Department – Golestan general hospital, Ahvaz Jundishapur University of Medical Sciences. Ahvaz Iran Tel: 986133743057

Dr Ali Kisat :E-mail address:Kiasatali@yahoo.com

Nuclear Medicine Department – Golestan general hospital, Ahvaz Jundishapur University of Medical Sciences. Ahvaz Iran Tel: 986133743057

Behrooz Etesami-mail address: Etesamibehrooz@gmail.com

Nuclear Medicine Department – Golestan general hospital, Ahvaz Jundishapur University of Medical Sciences. Ahvaz Iran Tel: 986133743057

Dr Mostafa Erfani : E-mail address; mgandomkar@acoi.org.ir

Radiation Application Research School, Nuclear Science and Technology Research Institute, [NSTRI], Tehran, Iran

Behzad Norouzi: E-mail address; ph.behzad@yahoo.com

School of pharmacy ,Ahvaz Jundishapur University of Medical Sciences ,Ahvaz ,Iran

Mohammad Hadi Baghersad : E-mail address ; Hadi2064@yahoo.com

Applied Biotechnology Research Center ,Baqiyatallah University of Medical Sciences ,Tehran ,Iran

Type of article: Original article

Objective

The main aim of this investigation is the clinical application of Ultrasound irradiation technique as an alternative method to reconstitute sestamibi kits in comparison of water boiling bath method.

Methods

The740-3700 MBq (20-100 mCi) ^{99m}Tc-MIBI (Sestamibi) complex samples were prepared due to ultrasound irradiation technique or boiled water bath method as a standard method. Twenty patients (8 men and 12 women; age range 30-72, median 52.45years) have been referred to Golestan hospital for myocardial perfusion imaging (MPI). The subjects have been divided randomly into group A (3 men, 7 women, age rang 36-67, median 51.7 years) and group B (5 men, 5 women, age range 30-72, median 50.3 years) respectively. The ^{99m}Tc-MIBI radiopharmaceuticals have been prepared by Ultrasound irradiation technique administrated to group A and ^{99m}Tc-MIBI complex samples due to the boiled water bath technique administrated to the other group. For all patients the protocol two day stress/rest MPI was performed.

Results

The radio-HPLC and TLC studies have indicated that the ^{99m}Tc-MIBI complex samples with good yields could be prepared successfully due to new developed technique. The scintigraphy imaging studies have demonstrated the ^{99m}Tc-Sestamibi prepared due to above mention modalities very identical biodistribution in the heart, thyroid, lung, liver, gallbladder, kidneys, stomach, large intestine and bladder of the subjects. Any unexpected accumulation of radiotracer samples have not observed in our approach.

Conclusions

The ultrasound irradiation technique is convenient and sufficient method to prepare ^{99m}Tc-Sestamibi .It can be recommended as an alternative method to reconstitute Sestamibi kits particularly in emergency situations in order to reduce potentially medical risk by avoiding any delay in acute therapy for myocardial infarction.

Key words: Sestamibi, ^{99m}Tc , ^{99m}Tc-MIBI, Ultrasound Irradiation

Introduction

Technetium 99m 2-methoxy isobutyl isonitirle (^{99m}Tc-MIBI) is a lipophilic cation complex that has been accumulated in viable myocardial tissue by passive diffusion into myocyte with subsequent binding to the mitochondria within the cell. This radiotracer has been demonstrated suitable characteristics for myocardial perfusion studies [1-6].However this radiopharmaceutical has been suggested to evaluate tumors of breast [7, 8], parathyroid [9 -11] and lung cancer [12-14].The frizzed –dried kit of sestamibi contains 2-methoxy isobutyl isonitirle (Sestamibi) as a performed copper (I) complex, Cu $(MIBI)_4^+$ BF_4^-[tetrakis (2-methoxy isobutyl isonitirle) Cu (I) tetrafluroroborate], which facilitates labeling by ligand exchange at elevated temperature. Reconstitution the MIBI kit with sodium 99mTc pertechnetate ($Na^{99m}TcO_4$) is performed according to the instructions provided by the manufactures. The volume 1-3ml of freshly eluted $Na^{99m}TcO_4$ 925-5550 MBq (25-150 mCi) is added to the MIBI vial aseptically. The shielded vial must be agitated vigorously to dissolve the vial contents. Then the vial is put in a boiling water bath for 10 min. After heating, the vial is placed into the lead shield and cooled at room temperature for approximately 15 min. The labeling process of MIBI as a conventional method is time-consuming. The microwave heating technique has been introduced as an alternative method for labeling of MIBI by $Na^{99m}TcO_4$.The new developed modality has been suggested not only for synthesis of MIBI [15],but also for labeling of MIBI by $Na^{99m}TcO_4$[16,17].

The time for labeling of MIBI was reduced to 10 seconds due to the microwave heating technique. In spite of rapid preparation ^{99m}Tc-MIBI complex by microwave oven facility, this method has following precaution factors that must be considered. Geometry of samples inside the microwave oven is important. There is a potentially risk of sparking for the presence of metal

cap of the vial. Any residual gas left in the head space of the vial could cause an ejection of the rubber stopper due to the excess steam pressure built up the vial. Microwave ovens with digital control panel are more suitable for setting short heating time (i.e.10 seconds) since they can be accurately set at the required heating period .The loss or variation of microwave power output and frequency related to extended use of the microwave oven should be evaluated on a long term usage. Finally any technical error in setting the instrument heating time below or beyond the predetermined time that may result in the ^{99m}Tc-MIBI solution being rendered unsuitable for clinical use[17]. Therefore, this new developed modality is not common use to prepare ^{99m}Tc-MIBI complex samples in nuclear medicine departments practically. Ultrasound irradiation technique has widely used in chemistry [18-21].This procedure is more convenient and can be carried out in higher yields, shorter time or milder conditions under ultrasound irradiation. According to literature, an experiment has been performed and reported 37 MBq (1mCi) ^{99m}Tc-MIBI complex samples with suitable yield due to ultrasound irradiation method and investigated the biodistribution ^{99m}Tc-MIBI complex in the rat's heart in comparison to the ^{99m}Tc-MIBI complex which prepared with water bath method [22].

The main propose of this study is clinical application ultrasound irradiation technique to prepare ^{99m}Tc-MIBI complex and biodistribution in the heart in comparison the ^{99m}Tc-MIBI complex which prepared due to the water bath method.

Materials and Methods

All chemical materials have been purchased from Merck or Fluka .The chemicals and solvents were of the highest purity and analytical grade and used without further purification. The freeze-dried kits of MIBI and ^{99}Mo/^{99m}Tc generator have been provided by Radioisotope Division of

Atomic Energy Organization of Iran (AEOI) .Technetium-99m as sodium pertechnetate was obtained from an in-house $^{99}Mo/^{99m}Tc$ generator using 0.9% saline.

Radiopharmaceutical preparation and quality control

The preparation of ^{99m}Tc-MIBI complexes were following according to manufacturer's instructions as a standard method or due to new developed technique. Technetium-99m as sodium pertechnetate (Na $^{99m}TcO_4$) was obtained from an in-house $^{99}Mo/^{99m}Tc$ generator using 0.9% saline. Commercial MIBI kits (AEOI, Iran) were used.

The740(n=10),1480(n=4),2220(n=2),2960(n=2) and 3700 MBq (n=2) 20,40,60,80and100 mCi freshly eluted Na $^{99m}TcO_4$ in maximum 2 ml saline were added to MIBI kits and put in the lead shields. The shielded vials were shaked for 30 seconds and mixture heated on a boiling water bath for 10 minutes, according to the protocol provided by the manufacturer (as a standard method) or the vials were sonicated in the thermo stated bath (Elma, P =95 w made in Germany) at 65 °C for 1 minute (as a new developed method).The radiochemical purity and labeling efficiency of ^{99m}Tc-MIBI complexes were determined by ascending Instant Thin-layer (ITLC) chromatography and Radio- High Performance Liquid Chromatography (Radio-HPLC). ITLC was developed by using Whatman No.3 filter paper chromatography as the stationary phase. As the mobile phase, two kinds of solvents were used, physiological saline solution and methanol. Standard 10 cm in length and 2 cm in width strips were used for each chromatogram. The strips were marked with pencil 1 cm (origin) and 0.5 cm (solvent front) from the base .A single spot of ^{99m}Tc-MIBI complex was applied with a capillary pipette on the 1 cm line. The strips were allowed to dry at the room temperature and then placed in air-tight containers.

In radiopharmaceutical analysis study by ITLC using normal saline as the mobile phase, ^{99m}Tc-MIBI complex and reduced ^{99m}Tc at the point of spotting, while free $^{99m}TcO_4^-$ moved to the

solvent front. By using methanol as another mobile phase ^{99m}Tc-MIBI complex and $^{99m}TcO_4^-$ moved to the solvent front, whereas reduced ^{99m}Tc remained at the point of spotting.

After migration of the mobile phase one centimeter from the top, the strips were dried and cut to ⅓ lower and ⅔ upper pieces. Each piece was counted using a single channel counter with NaI (Tl) detector. For finally quality control of all ^{99m}Tc-MIBI complexes was performed with analytical reverse-phase RP-HPLC on a JASCO 880-PU intelligent pump HPLC system (Tokyo, Japan) equipped with a multiwave length detector and a flow-through Raytest-Gabi g-detector CC 250/4.6 Nucleosil 120-5 C-18 column from Teknokroma was used for HPLC. For radionuclide analysis of ^{99m}Tc-MIBI complex by HPLC a volume of 10 µl of the test solutions were injected into the C-18 reverse phase column and 0.1 % trifluoroacetic acid /water (solvent A) and acetonitrile (solvent B) were used as a mobile phase in following gradient:0 min 95% A(5% B), 5 min 95% A (5% B) ,25 min 0 % A (100% B) ,30 min 0 % A (100 % B), flow =1 ml/min Fig 1.

Please consider Fig 1 here

Patients

Twenty patients (8 men and 12 women; age range 30-72, median 52.45years) have been introduced to Golestan hospital for myocardial perfusion imaging (MPI). The patients with history of renal failure or liver disease were excluded from this study. The patients have been divided randomly into two groups. Group A and B included 10 patients (3 men, 7 women, age rang 36-67, median 51.7 years), (5 men, 5 women, age range 30-72, median 50.3 years) respectively. The ^{99m}Tc-MIBI radiopharmaceuticals have been prepared by Ultrasound

irradiation technique administrated to group A and ^{99m}Tc-MIBI complex samples due to the boiled water bath technique administrated to the other group. This approach was approved by the ethics committee of our University. All subjects signed an informed consent form based on the guidelines of this committee before participation in the investigation.

Myocardial perfusion imaging

A two day stress/rest MPI protocol was performed on all patients. The555-740 MBq (15-20 mCi) ^{99m}Tc-MIBI radiotracers have been injected intravenously to the subjects at the stress and the same amount at the rest on the following day. Pharmacologic stress was obtained by intravenous infusion of 0.56 mg per kilogram of body weight of dipyridamole in 20 ml normal saline over 4 minutes under electrographic monitoring. The ^{99m}Tc-Sestamibi radiopharmaceutical was administered 3 minutes later. The exercise stress protocol included a step-wise increase in heart rate to reach the age dependent target heart rate. Exercise was considered inadequate if the patient achieved less than 85 % of the expected heart rate depending on the age or an ischemic ST-segment depression. ^{99m}Tc-MIBI radiopharmaceutical was injected intravenously at peak exercise and exercise continued at the same level for an additional one to two minutes after administration of radiotracer .Rest phase study was performed on the following day by the same protocol except imaging was performed 60 minutes following radiotracer administration. The images have obtained 1-2 hours after radiotracer injection when the subjects in the supine position. Scintigraphy imaging study was obtained over 180 ° orbits from right anterior oblique 45° (RAO) to left posterior oblique 45°(LPO) using a single-head gamma camera (E-Cam , model 2001 Siemens USA) equipped with high-resolution collimator. Acquisition parameters for myocardial and whole body scan were as follows. A- myocardial image: 64×64 matrix, zoom ×1.5, and energy window 140 keV, counter clockwise rotation, start angle 45 degree, 100 kilo

counts in first frame (first view by count), RAO to LPO direction, reconstruction method: filter back projection, Butterworth filter (cut off frequency 0.4 cycles/pixel and power 5).Reconstruction images were exhibited in short axis, vertical long axis and horizontal long axis. The total acquisition time was approximately 15 minutes. B-whole body image has been obtained 1-2 hours after radiotracer injection with velocity of 12 cm/min and matrix size: 256×1024. For image acquisition, a 20 % acceptance window around the 140 keV photo peak was used. Two criteria at the rest phase were used for interpretation of MIBI scans. First by dividing the counts in heart to total body by using available commercial software, the cardiac uptake to whole body uptake was calculated for each subject. Therefore, in whole body radioisotope scanning region of interest (ROI) was generated around cardiac uptake and then second ROI was generated around whole body uptake in anterior views. The accumulation of radiotracer in heart to whole body was measured. The background subtraction was not used.

Please consider figure 2 here

Second visual score (0, +, ++, +++) was used. The scores ++ equal uptake of radiotracer to heart, the scores +++ uptake greater than heart, the scores + less uptake than heart and finally 0 equivalent no uptake were chosen. The distribution of radiotracer in heart, thyroid, lung, liver, gallbladder, kidneys, stomach, large intestine and bladder were considered.

Please consider table 1 here

Scintigraphy images were interpreted by three independent experienced nuclear physicians and their final opinion was achieved by consensus. The observers were unaware of the process of preparation ^{99m}Tc-MIBI complex samples.

Results

Radio labeling procedure with ^{99m}Tc can yield two main radiochemical impurities, $^{99m}TcO_4^-$ and $^{99m}TcO_2$. The yield of ^{99m}Tc-MIBI complex samples which prepared by boiling water bath as a standard method were 95±1.8 %(n=20). The radio labeling yield of ^{99m}Tc-MIBI complex samples which reconstituted due to ultrasound irradiation technique as an alternative method were 92.5±3% (n=20) .The retention times of $^{99m}TcO4^-$ and ^{99m}Tc-MIBI complex were approximately 3.2 and 20.4 minutes respectively. The HPLC retention time of ^{99m}Tc-MIBI complexes which were prepared due to the above mention methods were identical (figure 1). This finding demonstrates the successful preparation of ^{99m}Tc-MIBI complexes due to ultrasound irradiation method. A labeling efficiency of more than 90 % was necessary for the intravenous injection of the radiotracer samples. All the patients referred to our center for routine myocardial perfusion imaging were eligible. The patients have been entered in this investigation monitored for two months after experiment. During this study none of the patients reported any adverse reaction like nausea, vomiting, diarrhea, bronchospasm, hypotension, hypertension, tachycardia, bradycardia, fever, chills, muscle cramps and allergic reaction after the ^{99m}Tc-MIBI radiopharmaceutical injection. The images demonstrated suitable uptake of radiotracer samples in the heart for each case. The quality of images in the both groups A and B were similar. For this reason it has provided for us, MPI studies performed for the subjects participated in the group A like the other group (figure3).

Please consider Figure 3 here

Whole body scan has been performed 1 to 2 hours after the ^{99m}Tc-MIBI radiotracer administrated intravenously at the rest phase. The ^{99m}Tc-MIBI samples were prepared by ultrasound irradiation or boiled water bath techniques shown identical biodistribution in the heart of patients. The accumulations of ^{99m}Tc-MIBI radiotracers prepared by above two mentioned modalities were approximately 2% in the patients' heart (figure 2). The biodistribution of radiotracer samples in other organs such thyroid, lung, liver, gallbladder, kidneys, stomach, large intestine and bladder were similar (table 1) .We have not observed any unexpected finding of accumulation radiotracer samples in the patients.

Discussion

Cationic Tc (I)-hexakis (2-methoxy isobutyl isonitirle) tetrafluroroborate is labeled by reacting tetrakis (2-methoxy isobutyl isonitirle) copper (I) tetrafluroroborate adduct with freshly eluted solution of 99mTc pertechnetate by using the kit formulation. Heating the content vial in boiling water or by any other modalities is the pivotal role for formation ^{99m}Tc-MIBI complex. The exact structure of ^{99m}Tc-MIBI complex has not been elucidated .It has been suggested hexakis (alkylisonitirle) technetium (I) complex is monovalent cation with a central Tc (I) core surrounded by six identical lipophilic ligand coordinated through the isonitirle carbon [23].

The suitable conditions for the preparation of ^{99m}Tc-MIBI complex samples under ultrasound irradiation technique have been reported [22]. In new developed technique, local induced heating due to ultrasound irradiation itself without heating to 65 °C was not efficient to prepare radiotracer in sufficient yield. Isonitirle as an electron donor group in sestamibi is coordinated with technetium. The nature of this kind of bonding is not strong as covalent bonding. Therefore, elevated temperature above 65°C for more than one minute could influence on the nature of bonding or degrade the structure of ^{99m}Tc-MIBI complex under ultrasound irradiation condition.

For this reason the yield of ^{99m}Tc-MIBI complex samples were decreased significantly. The previous assessment indicated that the volume of freshly eluted 99mTc pertechnetate sodium, temperature and time period of ultrasound irradiation, according to the power of ultrasound instrument facility were the most important factors to prepare the ^{99m}Tc-MIBI complex samples with sufficient yields. The Radio-HPLC and TLC analysis studies have been shown the successful reconstitution sestamibi kits by new developed modality. ^{99m}Tc-Sestamibi is a lipophilic, monovalent cation that localizes in the myocardial cells by simple diffusion without active transport .It has been proposed sestamibi molecule to bind to a small molecular weight cytosolic protein [24].Unlike thallium 201 it does not redistribute over time. Therefore, separate injections have to be administrated intravenously to patient for stress and resting studies .The investigation of the patients did not demonstrate considerable differences in biodistribution of radiotracer samples in both the stress and rest phases which prepared due to ultrasound or boiled water bath techniques in this approach. Visual analysis of images revealed a good image quality in each case. The outcome of our investigation has been demonstrated the preparation of ^{99m}Tc-MIBI complex samples by ultrasound irradiation technique is reliable and reproducible method to facilitate the reconstitution ^{99m}Tc-sestamibi kits. The preparation of ^{99m}Tc-MIBI complex by new developed method has the following advantages. The time labeling of sestamibi by pertechnetate sodium by ultrasound irradiation method versus to water bath method was reduced considerably. The ^{99m}Tc-MIBI complex samples could prepare in sufficient amounts with good yields. The geometry of samples in ultrasound device was not important factor. There is no potentially risk of sparking for the presence of metal cap of the vial inside the ultrasound apparatus. In addition to the above mentioned factors, the potentially risk of absorbed ionization irradiation to the personals of nuclear medicine department could be decreased significantly. The

suggested technique for preparation ^{99m}Tc-MIBI complex can be performed in any nuclear medicine centers and permitted a fast and reliable method to make ^{99m}Tc-MIBI complex available for either routine or emergency use. To achieve this propose, it is necessary to set out this technique in nuclear medicine departments in order to find out the suitable conditions from the aspect of temperature and time period of ultrasound irradiation according to the power of instrument.

Conclusion

The ^{99m}Tc-MIBI complex can be prepared rapidly and efficiently by the ultrasound irradiation technique. This achievement may be decreased potentially risk to the patient by refraining any delay in emergency situation like acute therapy especially for myocardial infarction patients. Green chemistry can open a new field in nuclear medicine. Ultrasound irradiation technique can be suggested for preparation the radiopharmaceuticals which the reconstitution of kits are time consuming.

References

1.Beller GA ,Watson DD. Physiological basis of myocardial perfusion imaging with the technetium99m agents . Semin Nucl Med 1991;21:170-172

2.Taillerfer R ,Lambert R ,Essiambre R ,Phaneuf DC ,Leveille J .Comparison between thallium-201,technetium-99m-sestamibi and technetium-99m-teboroxime planar myocardial perfusion imaging in detection of coronary artery disease. J Nucl Med 1992;33:1091-1098

3. Manka-Waluch A, Palmedo H ,Reinhardt MJ ,Joe A ,Manka C ,Guhlke S ,et al. Myocardial uptake characteristics of three 99mTc-labeled tracers for myocardial perfusion imaging one hour after rest injection. Ann Nucl Med 2006 ;20:663-670

4. Faerd-Esfahani A ,Fallahi B ,Mohaghegh A ,Assadi M ,Beiki D ,Eftekhari M ,et al . The value of myocardial perfusion imaging with Tc-99m Mibi for the prediction of perfusion improvement after percutaneous transluminal coronary angioplasty. Iran J Nucl Med 2010;18:7-13

5.Fukushima K ,Mamosa M ,Kondo C ,Higuchi T ,Kuasakabe K ,Hagiwara N. Myocardial 99mTc-sestamibi extraction and washout in hypertensive heart failure using an isolated rat heart. Nucl Med Biol 2010;37:1005-1012

6. Piwnica-Worns D ,Kronauge JF ,Holman BL ,Davison A ,Jones AG . Comparative myocardial uptake characteristics of hexakis(alkylisonitrile) technetium (I) complexes,effect of lipophilicity .Invest Biol 1989;24:25-29

7. Vecchio SD ,Salvatore M. ^{99m}Tc-MIBI in the evaluation of breast cancer biology . Eur J Nucl Med Mol Imaging 2004;31:88-96

8. Ho park Y ,Ferrante J ,Robinson RE ,Arvay K .Occult breast cancer detection with technetium-99m-sestamibi .J Nucl Med Technol 1999;27:298-300

9. Spanu A , Falchi A ,Manca A ,Marongiu P ,Cossu A ,Pisu N ,et al . The usefulness of neck pinhole spect as a complementary tool to planar scintigraphy in primary and secondry hyperparathyroidism. J Nucl Med 2004;45:4048

10. Arsalan N ,Ilgan S ,Urhan M ,Karacalioglu AO ,Arsalan I ,Ozturk E ,et al . The role of technetium -99m-sestamibi parathyroid scintigraphy in the detection and localization of parathyroid adenomas in patients with hyperparathyroidism: comparison with technetium – thallium subtraction scan and ultrasonography. Turk J Endocrinol Metab 1999;2:47-52

11.Digonnet A ,Carlier A ,Willemse E ,Quiriny M ,Dekeyser C ,Aubain NS ,et al. Parathyroid carcinoma :review with three illustrative cases. J cancer 2011;2:532-537

12.Zhou J ,Higashi K ,Ueda Y ,Kodama Y ,Guo D ,Jisaki F ,Sakura A ,Takegami T ,Katsuda S ,Yamamoto I .Expression of multidrug resistance protein and messenger RNA correlate with ^{99m}Tc-MIBI imaging in patients with lung cancer .J Nucl Med 2001;42:1476-1483

13.Dirlik A ,Burak Z ,Goksel T ,Erinc R ,Karakus H ,Ozcan Z ,et al .
The role of Tc-99m sestamibi in predicting clinical response to chemotherapy in lung cancer. Ann Nucl Med 2002;16:103-108

14. Akgun A ,Cok G ,Karapolat I ,Goksel T ,Burak Z .Tc-99m spect in prediction of prognosis in patients with small cell lung cancer . Ann Nucl Med 2006;20:269-275

15. Lima MJC ,Marques FLN ,Okamoto MRY ,Garcez AT ,Sapienza MT ,Buchpigqel CA. Preparation and evaluation of modified composition for lyophilized kits of $[Cu(MIBI)_4]BF_4$for [^{99m}Tc]technetium labeling. Braz Arch Biol Technol 2005;48:1-8

16. Gagnon A ,Taillerfer R ,Bavaria G ,Leveille j. Fast labeling of technetium-99m-sestamibi with microwave oven heating. J Nucl Med Technol 1991;19:90-93

17. Hung JC ,Wilson ME ,Brown ML ,Gibbons RJ . Rapid preparation and quality control method for technetium-99m-2-methoxy isobutyl isonitirle(Technetium-99m-sestamibi). J Nucl Med 1991;32:2162-2168

18. Khalaj A ,Doroudi A ,Adibpour N ,Araghi M .N-alkylation and N-acylation of 2,4dinitrophenylamine by ultrasound irradiation .Asian J Chem 2009;21:997-1001

19. Singh AK ,Shukla SK ,Quraishi MA .Ultrasound mediated green synthesis of hexa-hydro triazines. J mater Environ Sci 2011;2:403-406

20. Jiang W ,Zhu W ,Jiang C ,Xuan S ,Gong S ,Zhang Z .The controllable synthesis of nanoporous $SrTiO_3$ by an ultrasound irradiation approach. Smart Mater Struct 2011;20:1-5

21. Du C , LI JT. Synthesis of 1,5diaryl-1,4pentadien-3-one amidohydrazone hydrochloride under ultrasound irradiation . Eur J Chem 2012;9:2108-2113

22. Doroudi A ,Saadati SM ,Hassanpour H ,Ahmadi F ,Erfani M ,Rezaee S ,et al. Preparation of ^{99m}Tc-MIBI under ultrasound irradiation .J Radioanal Nucl Chem 2013;298:1185-1190

23. Piwnica-Worms D , Kronauge JF , Chius ML .Uptake and retention of hexakis (2-methoxyisobutyl isonitirle) technetium (I) in cultured chick myocardial cells .Circulation 1990;82:1826-1838

24. Mousa SA ,Williams SJ .Myocardial uptake and retention of Tc-99m hexakis-aliphatic isonitirles: evidence for specificity [abstract]. J Nucl Med 198;27:995

Fig1. Radio-HPLC chromatograph of ^{99m}Tc-MIBI complexes were prepared by ultrasound irradiation technique (a) versus boiling water bath method (b) 30 min post reconstitution. The retention times of $^{99m}TcO4^-$ and ^{99m}Tc-MIBI complex were approximately 3.2 and 20.4 minutes respectively.

Fig2. Whole body radioisotope images patients have been performed in the rest phase after the 555-740 MBq (15-20 mCi) ^{99m}Tc-MIBI administrated intravenously. By using available commercial software, the accumulation ratio of radiotracer in the patient's heart to whole body was calculated by dividing the activity of heart to total body counts. The uptakes of ^{99m}Tc-MIBI

in the heart to the total body were approximately 2 %. The ^{99m}Tc-MIBI complex samples were prepared under a: ultrasound irradiation b: boiled water bath conditions.

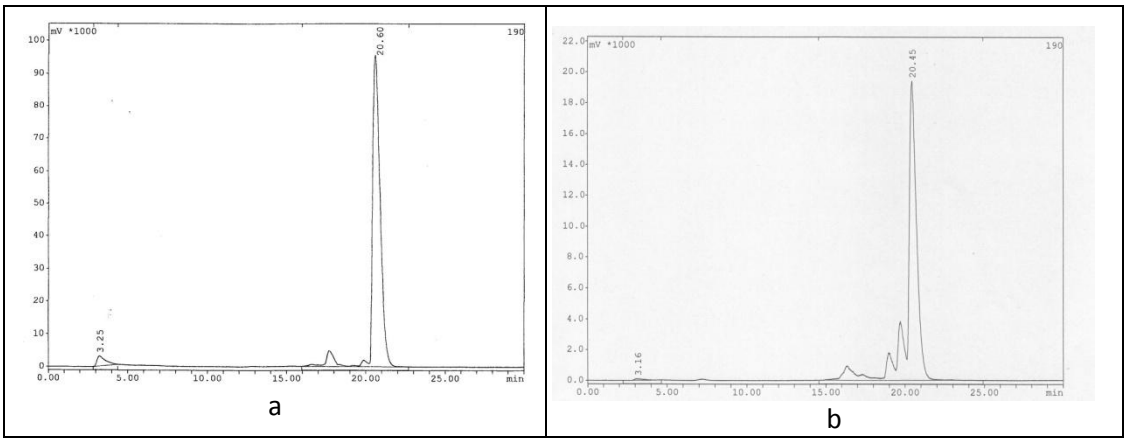
Fig3. Single photon emission computed tomography perfusion images of the same patients were acquired with the ^{99m}Tc-MIBI radiopharmaceutical samples. A two day stress/rest myocardial perfusion imaging protocol was performed .The short axis (top 2 rows), vertical long-axis (middle 2 rows) and horizontal long-axis (bottom 2 rows) slices were displayed at stress and rest. The images in first row of short axis, vertical long-axis and horizontal long- axis belong to the stress phase of myocardial perfusion imaging. The camera rotation around the patient's chest from 45° degrees right anterior oblique (RAO) to 45° degrees left posterior oblique (LPO). The images were obtained 1-2 hours after the 555-740 MBq (15-20 mCi)^{99m}Tc-Sestamibi radiotracer samples administration intravenously, a: radiotracer sample reconstituted due to ultrasound irradiation technique, b: radiopharmaceutical sample prepared by boil water bath method.

Table1. Patient characteristics in both groups A and B have been shown in the rest phase after the 555-740 MBq (15-20 mCi) injected intravenously. The Radiotracer samples were reconstituted under ultrasound irradiation modality in the group A or boiled water bath method in the group B. The distribution of radiotracer samples in heart, thyroid, lung, liver, gallbladder, kidneys, stomach, large intestine and bladder were considered. The scores ++ equal uptake of radiotracer to heart, the scores +++ uptake greater than heart, the scores + less uptake than heart and finally 0 equivalent no uptake were considered.

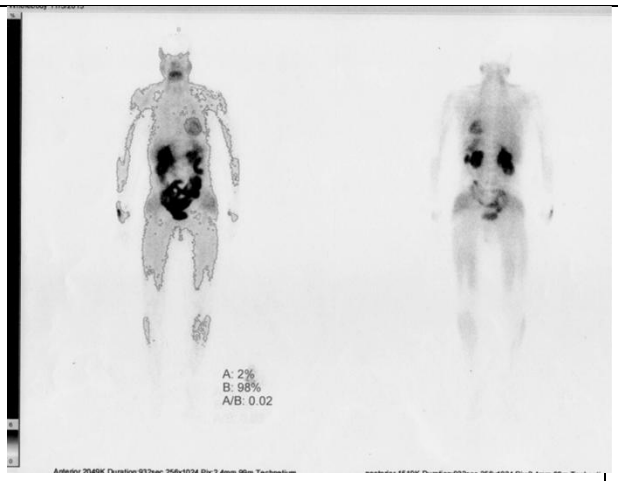
Acknowledgments

This approach is part of pharm-D thesis of Behzad Norouzi and especial thanks to Ahvaz Jundishapur University of Medical Sciences for the financial support and also to Radioisotope Division of Atomic Energy Organization of Iran for providing the freeze-dried Sestamibi kits and $^{99}Mo/^{99m}Tc$ generator.

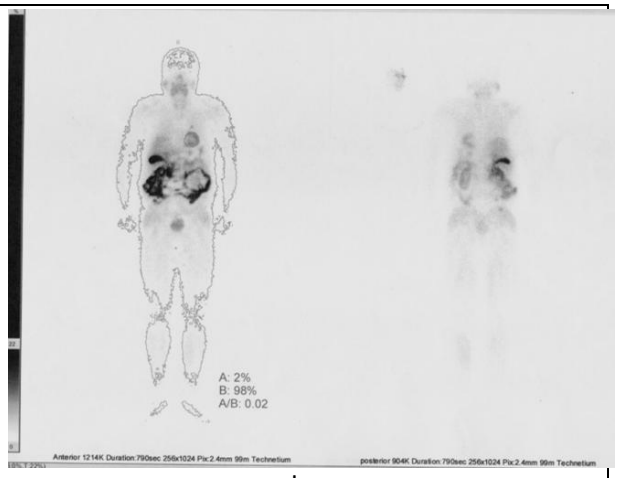
Figure


mV *1000
190
20.60
3.25
0.00
5.00
10.00
15.00
20.00
25.00
min
a
mV *1000
190
20.45
21.16
0.00
5.00
10.00
15.00
20.00
25.00
min
b

Figure

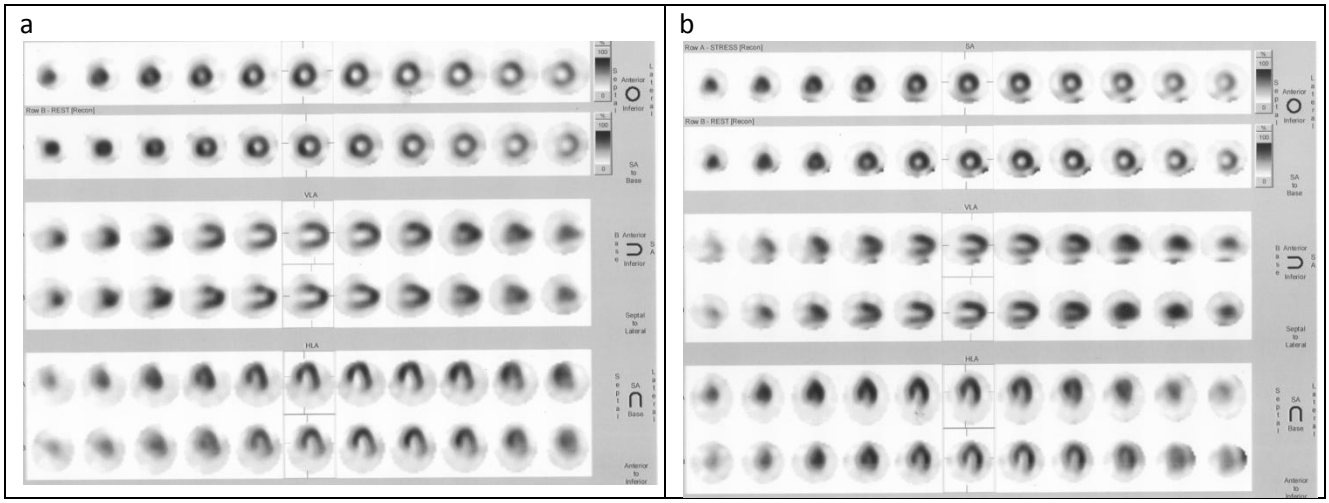

A: 2%
B: 98%
A/B: 0.02

a


A: 2%
B: 98%
A/B: 0.02
Anterior 1214K Duration:790sec 256x1024 Pix:2.4mm 99m Technetium
posterior 904K Duration:790sec 256x1024 Pix:2.4mm 99m Technetium

b

Figure


a
b
Row A - STRESS [Recon]
Row B - REST [Recon]
SA
VLA
HLA
Anterior
Inferior
SA to Base
Septal to Lateral
Anterior to Inferior

Table

Age	Sex	Group	Cardiac Uptake%	Thyroid	Lung	Liver	Gallbladder	Stomach	Large intestine	Kidneys	Bladder
46	F	A	2	0	0	++	+++	0	+++	++	+++
47	F	A	3	+	0	++	++	0	+++	+++	++
46	F	A	2	+	0	+	+++	+	+++	++	+++
40	F	A	1	++	0	++	+++	0	+++	+++	++
36	F	A	2	++	0	++	++	0	+++	+++	+++
66	M	A	2	+	0	++	+++	0	+++	++	++
51	F	A	3	+	+	+	+	0	+++	+	+
63	M	A	2	0	+	+	+++	0	+++	+++	++
67	F	A	1	0	0	+	+++	0	+++	++	+++
55	M	A	4	0	+	++	+	0	+++	+++	+++
47	F	B	2	0	0	+	+++	0	+++	+++	+++
38	M	B	3	+	+	+	+++	0	+++	+++	+++
72	M	B	2	0	0	+	+++	0	+++	++	+++
61	F	B	1	++	0	++	+++	0	+++	+++	+++
65	M	B	2	+	0	+++	+	0	++	+++	+++
43	F	B	2	+	+	+++	+	0	+++	+++	+++
54	M	B	1	0	0	++	+++	0	+++	+++	+++
65	F	B	2	++	0	+++	++	0	+++	+++	+++
57	M	B	2	+	0	++	+++	0	+++	+++	+++
30	F	B	2	++	0	+++	+	0	+++	+++	+++

Annals of Nuclear Medicine

Certification Form:

Conflict of Interest Acknowledgment, Certification of Coauthors, and Exclusive Publication Statement

Manuscript title: New Technique for Preparation of ^{99m}Tc-MIBI Complex

The first author, signing on behalf of all coauthors of this article, attests that all of the coauthors have contributed significantly to and share in the responsibility for the release of any part or all of the material contained within the article named above. The undersigned certifies that (1) this material is new, original, and has not been submitted to another publication for concurrent consideration, (2) the author warrants that he/she has obtained any necessary permissions for re-use of copyrighted figures or other material, (3) all human and/or animal studies undertaken as part of the research from which this manuscript was derived are in compliance with the pertinent regulations of the institution(s) involved and with generally accepted guidelines governing such work, and (4) all financial or other relationships that could be construed as a potential conflict of interest have been disclosed using the particular form (Form 2, which is posted on the ANM web site) at the time of submission, and all sources of financial support for this study have been specified and noted in the acknowledgments.

In cases where no regulated conflict of interest exists for all coauthors, the corresponding author may so declare on behalf of all coauthors. In addition, wording such as "No potential conflicts of interest were disclosed" is inserted in the Acknowledgements section of the article.

☐ I certify that no conflict of interest exists in relation to this article. (*Please indicate with a checkmark in the box.*)

Upon publication, author(s) will be charged as follows:

Additional Page Charge: Papers no longer than four printed pages (including figures and tables) are published free of charge. Charges for additional pages and plate making for figures will be sent to the principal author. An estimate of the approximate charges may be provided upon the author's written request.

Color in Print: Authors are responsible for the costs of color reproduction (€ 950 per article).

Charge for English Editing: At the editor's discretion, accepted manuscripts may be copy-edited by Prof. John Gelblum for a charge of ¥ 2,000 per page.

Alireza Dorodi

First Author

28/7/2014

Printed Name/Date

This agreement must be signed by the first author, on behalf of all coauthors of this article, for the manuscript to be published.