International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.11, No.02, pp 156-167, 2018

The Preparation of ^{99m}Tc-MAG$_3$ radiotracer by Ultrasound

Alireza Doroudi[1]*, Sepide Ghasemi Falavarjani[1], Mohammad Javad Khodayar[2], Faramarz Ahmadi[3], Seyyed Mostafa Saadati[3], Ali Kiasat[3], BehroozEtesami[3], Mostafa Erfani[4]

[1]Medicinal Chemistry Department, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[2]Toxicology Department, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[3]Nuclear Medicine Department, Golestan General Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[4]Radiation Application Research School, Nuclear Science and Technology Research Institute (NSTRI), Tehran, Iran.

Abstract : The main aim of this investigation was to prepare ^{99m}Tc-MAG$_3$ samples under sonication. Then the radio complex samples were compared with the counterparts which prepared by boiling water bath as the standard method from the aspects of stability in normal saline, human serum, partition coefficient, protein bonding and finally the biodistribution in rat.The preparation of ^{99m}Tc-MAG$_3$ samples was examined to determine the ideal condition under sonication that radio complex samples could be prepared with appropriate radiochemical purity. The stability of radiotracer samples was assessed in normal saline up to 24 h post preparation. The partition coefficient, protein bonding and stability in human serum were analyzed. Then the biodistribution of radiotracer samples were evaluated in rat.The Radio-HPLC and ITLC assays indicated that the ^{99m}Tc-MAG$_3$ samples could be successfully prepared with suitable yields by sonication. The radiolabeling efficiency was above the 90% when the reaction was carried out at 60°C for 1 min. The radio complex samples showed good stability in normal saline and human serum. The partition coefficient and protein bonding were -2.4± 0.32 and 35.23± 1.15 respectively when the radiolabeling was performed by the standard method. These values were -2.51± 0.42 and 39.36± 1.7 when the radiolabeling was undertaken by sonication. The biodistribution of ^{99m}Tc-MAG$_3$ samples in the rats demonstrated that the radiolabeling procedure could not lead to a significant difference in the biodistribution of samples. The new developed technique can be recommended as an alternative for boiling water bath method to prepare ^{99m}Tc-MAG$_3$.
Keywords : Renal imaging, Sonication, ^{99m}Tc-MAG$_3$, Ultrasound radiation.

International Journal of PharmTech Research, 2018,11(2): 156-167.

DOI: http://dx.doi.org/10.20902/IJPTR.2018.11206

Introduction

The dynamic radioisotope renal imaging provides the important functional information to assist in the distinction of a variety of suspected genitourinary disorders. The basis of renogram curve is to analyse a series of images of the kidneys, as the radiotracer is removed from the blood, transited the kidneys and finally entered into the bladder [1,2].Renogram curves quantify the radiotracer movement through each kidney. The combination of renogram curves and dynamic images is used for the intellectual interpretation of renal scintigraphyimaging.Technetium99m diethylenetriaminopentaacetic acid (^{99m}Tc-DTPA) and technetium 99m mercaptoacetyltriglycine (^{99m}Tc-MAG_3) radiopharmaceutical agents are widely used for the dynamic renal scintigraphy in nuclear medicine [3].^{99m}Tc-DTPA was first suggested into clinical practice in 1970[4]. It has been remained as a radiotracer of choice for detection of urinary tract obstruction in nuclear medicine [5]. This radiotracer is a relatively small molecule which can be readily passed through the endothelial membrane. ^{99m}Tc-DTPA is filtered by glomerulus after intravenous injection. There is little or no secretion of ^{99m}Tc-DTPA radiotracer by the renal tubules and no notable amount of tubular reabsorption.^{99m}Tc-MAG_3 has been examined as a suitable candidate for ^{131}I-Hippuran for dynamic renography in nuclear medicine[6,7]. ^{99m}Tc-MAG_3 is rapidly excreted by the kidneys by glomerular filtration and active tubular secretion after intravenous injection. ^{99m}Tc-MAG_3 has a greater extraction fraction than ^{99m}Tc-DTPA which is three times higher than^{99m}Tc-DTPA and as a consequence giving a suitable target to background ratio[8]. Therefore, it provides the appropriate images in neonates, in patients with impaired function and in patients with suspected obstruction. The^{99m}Tc-MAG_3 clearance is correlated with the effective renal plasma flow (ERPF). Finally, the administered dose of^{99m}Tc-MAG_3 is less than ^{99m}Tc-DTPA in dynamic renal scintigraphy and consequently, the radiation dose to patients especially pediatrics and staff could be reduced [9-14]. The radiolabeling of ^{99m}Tc-DTPA is simply reconstituted by adding a sterile eluted solution of $^{99m}TcO_4$ to the freeze-dried kit in accordance with the manufacturer's product specification at the room temperature. But the preparation of ^{99m}Tc-MAG3 radiotracer is facilitated by heating and carried out at the high temperature due to the boiling water bath. Therefore, the radiolabeling of ^{99m}Tc-MAG3 versus ^{99m}Tc-DTPA is time-consuming. It is highly desirable the reaction of ligand and^{99m}Tc radionuclide is performed at the milder condition and shorter time for radiopharmaceutical work in nuclear medicine departments. Ultrasound irradiation technique is a branch of chemical science that the reactions are carried out under ultrasound waves. The frequency of waves is above 20 kHz that lie beyond the threshold of human hearing. Ultrasound waves cause cavitation phenomenon which generates enough energy to alter vibrational and rotational molecular states. The reactions under ultrasound waves can be leaded to better yields, faster rates and milder temperatures [15-18]. The new developed technique was examined to prepare the ^{99m}Tc-Sestamibi radiocomplex as an alternative and reliable method in clinical practice[19-21]. This approach was conducted to evaluate the application of ultrasound irradiation for the reconstitution MAG^3 freeze-dried kits by ^{99m}Tc radioisotope in comparison to the boiling water bath as a standard method.

Materials and methods

All chemical materials were procured from Merck and Sigma-Aldrich companies. The chemicals and solvents were the highest purity and analytical grade and used without further purification. The freeze-dried MAG_3 kits and ^{99}Mo/^{99m}Tc generators were supplied 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. The rats with 140±10 g were obtained from research center and experimental animal house of Ahvaz Jundishapur University of Medical Science. This study was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences. All the ethics issues were considered based on the Ahvaz Medical University of Medical Sciences (AMUP) on animal experiments. A total number of 10 adults, male NMRI were acclimated to the conditions for one week before the experiment. The animals were kept in individually cages in an air-conditioned room at 24±1ºC with a 12 hours' light-dark cycle and were fed with standard pellet diet and had free access to water. The rats were randomly assigned into two main groups equally.The radiotracer samples were administrated intravenously due to contra lateral tail. The radiotracer samples were prepared by the boiling water bath as conventional method administrated to the one group of rats. The radiotracer samples were prepared by the ultrasound irradiation as a new developed technique administrated to the other group of animals.

The radiolabeling of MAG3 by ^{99m}Tcand quality control

^{99m}Tc as sodium pertechnetate (Na $^{99m}TcO_4$) was obtained from an in-house ^{99} Mo/ ^{99m}Tc generator using 0.9% saline. The lyophilized MAG_3kits (AEOI, Tehran, Iran) were used. Radiolabeling and quality control procedure were undertaken on the basis of manufacturer's instructions. The lyophilized vials were taken from the freezer and put at the room temperature in order to reach the ambient temperature. After reaching the ambient temperature, half a milliliter of saline was incubated with a 2 ml syringe. After adjusting the size of the syringe, it entered the vial. The vial should contain a vacuum and the solution should be pulled in by itself. Then the 740MBq (20 mCi) freshly eluted $Na^{99m}TcO_4$ was added to the vial. The shielded vials were shaken gently for 5 min at the room temperature. The lead shield vials were heated by the boiling water bath apparatus at 100°C for 15 min or sonicated in the thermo stated bath (Elma, P= 95 W, made in Germany). The vials were incubated at the different times and temperatures in order to find out the optimum condition for the preparation of radiotracer samples with the sufficient yields under ultrasound waves. Instant thin layer chromatography on silica gel (ITLC-SG, Merck)and high performance liquid chromatography with gamma counter (Radio-HPLC) were used for quality control of radiochemical purities. Two different solvent systems were used as mobile phases in the ITLC analysis. The strips in 2 cm width and 10 cm length were used. The samples (2µl) were applied 1 cm from the bottom of strips. When the mixture of acetone and chloroform (80, 20) was used as mobile phase, the desired radio complex and reduced technetium99m ($^{99m}TcO_2$) remained at the spotting point while the free $^{99m}TcO_4$ traveled to solvent front. When the mixture of acetonitrile and normal saline (60,40) was used as another mobile phase, the $^{99m}TcO_2$ remained at the spotting point and radio complex and $^{99m}TcO_4$ moved to the solvent front. When the mobile phase was reached to 1 cm from the top of strips, they were removed from the air-tight containers and allowed to dry at room temperature in all studies. The strips were cut into ⅓ lower and ⅔ upper pieces. Each part was counted for 2 min under a single head camera equipped with low energy all-propose collimator using an energy centered a 140 keV with NaI (Tl) detector (Aktivimeter, Siemens, Germany).Each experiment was repeated three times and the mean yields of radio complex samples and radiochemical impurities shown in table 1.

Table 1: The 740MBq (20 mCi) freshly eluted solution of $Na^{99m}TcO_4$ was added to freeze-dried MAG_3 kits. The vials were sonicated in the thermo stated bath (Elma, P= 50 W, Germany) at 40,50,60 and 70 °C for 1,2,3,4 and 5 min. Each test was repeated three times and yields of $^{99m}TcO_4^-$, $^{99m}TcO_2$ and ^{99m}Tc-MAG_3 species respectively.

Temperature ℃	Time(Min)	$^{99m}TcO_4^-$%	$^{99m}TcO_2$ %	$^{99m}Tc-MAG3$ %
40	1	19.42± 0.15	20.49± 0.28	60.09± 0.12
	2	17.01± 0.17	17.68± 0.13	65.31± 0.31
	3	10.51± 0.11	15.83± 0.11	73.66± 0.26
	4	8.01± 0.2	14.34± 0.25	77.65± 0.16
	5	6.67± 0.36	15.15± 0.12	76.18± 0.13
50	1	9.81± 0.27	13.58± 0.33	76.61± 0.21
	2	6.68± 0.13	10.51± 0.16	82.81± 0.14
	3	5.29±0.18	9.46± 0.1	85.25± o.25
	4	3.54± 0.21	10.22± 0.22	86.24± 0.1
	5	2.03± 0.4	8.65± 0.19	89.32± 0.13
60	1	2.42± 0.14	5.27± 0.29	92.31± 0.17
	2	3.03± 0.18	6.01± 0.22	91.14± 0.15
	3	3.67± 0.21	7.04± 0.14	89.29± 0.3
	4	5.01± 0.35	8.28± 0.44	86.71± 0.21
	5	9.83± 0.11	11.12± 0.25	79.05± 0.35
70	1	3.37± 0.16	8.85± 0.13	87.78± 0.14
	2	5.81± 0.2	8.15± 0.11	86.04± 0.22
	3	7.00± 0.23	11.04± 0.19	81.96± 0.24
	4	6.41± 0.1	11.29± 0.13	82.30± 0.31
	5	10.78± 0.18	18.11± 0.22	71.11± 0.11

The yield of radio complex was increased from 60.09± 0.12% at 40 °C to 92.31± 0.17% at 60 °C. If the reaction temperature was increased from 60°C to 70°C, the efficiency of radiolabeling was dropped to less than 90%. The reaction time for producing radio complex samples was also important factor. As the reaction time increased from 1 min to 5 min at 60° C, the radiolabeling efficiency was reduced from 92.31± 0.17 to 79.05± 0.35%. Therefore, the temperature and reaction time were two crucial factors to prepare the ^{99m}Tc-MAG_3 radiotracer with the appropriate yields when sonication method was used. Due to the identification of the standard conditions for the reconstitution ^{99m}Tc-MAG_3 radiotracer samples by sonication, all reactions were performed at the above mentioned condition in order to continue this approach. The ITLC scanner profiles demonstrated the efficacy of radiolabeling ^{99m}Tc-MAG_3 radiotracer samples which were prepared by the boiling water bath or sonication methods. The high radiochemical purity in the two different solvent systems is shown in Fig1 and 2.

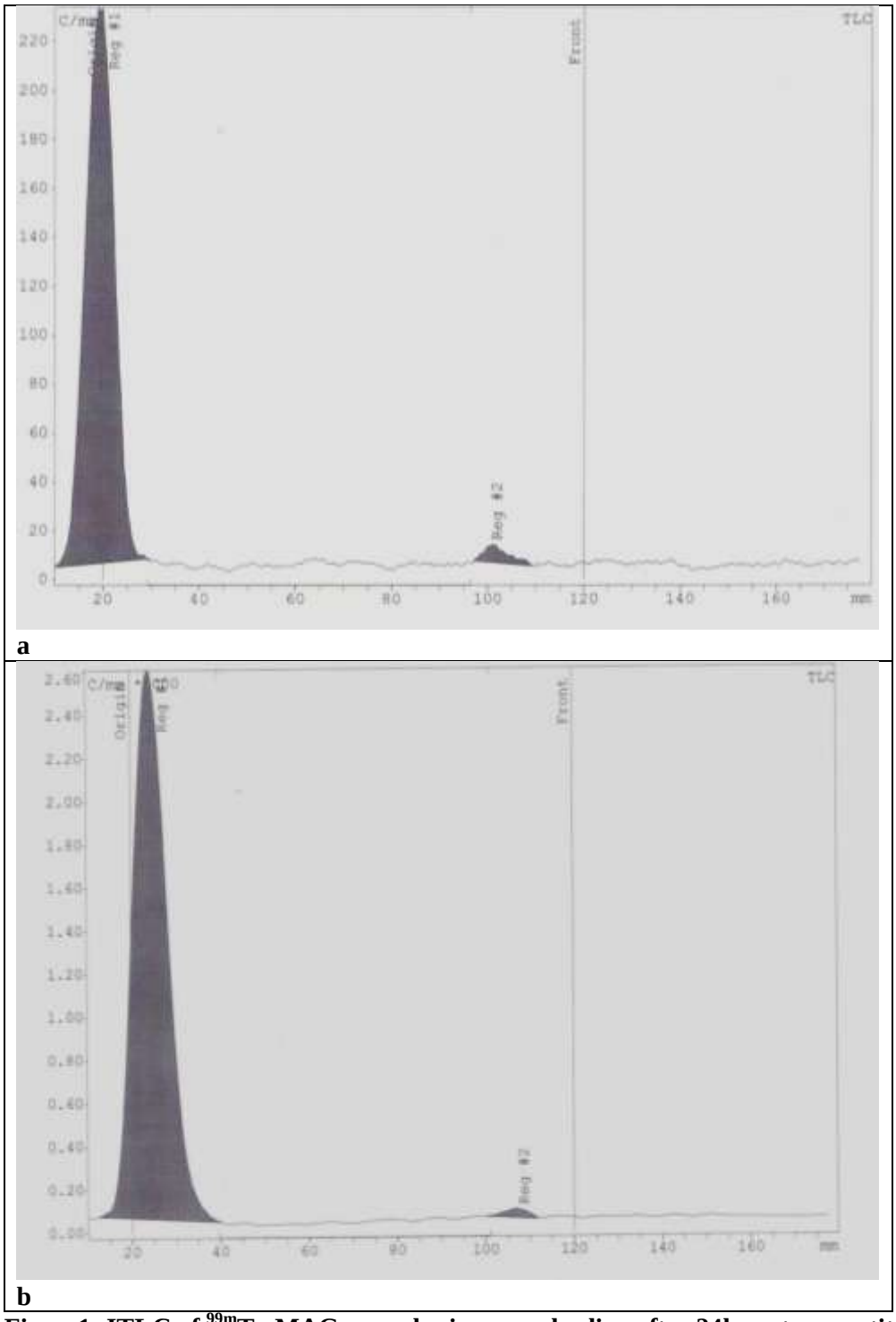


Figure1: ITLC of ^{99m}Tc-MAG_3 samples in normal saline after 24h post reconstitution.

Chromatograms on Whatman No:2 by using acetone 80%: chloroform20% as mobile solvent system. The R_f of $^{99m}TcO_2$, ^{99m}Tc-MAG_3 and free $^{99m}TcO_4$ are 0.00,0.00 and 0.7- 1. The radiotracer samples were prepared a: Boiling water bath, b: Ultrasound radiation.

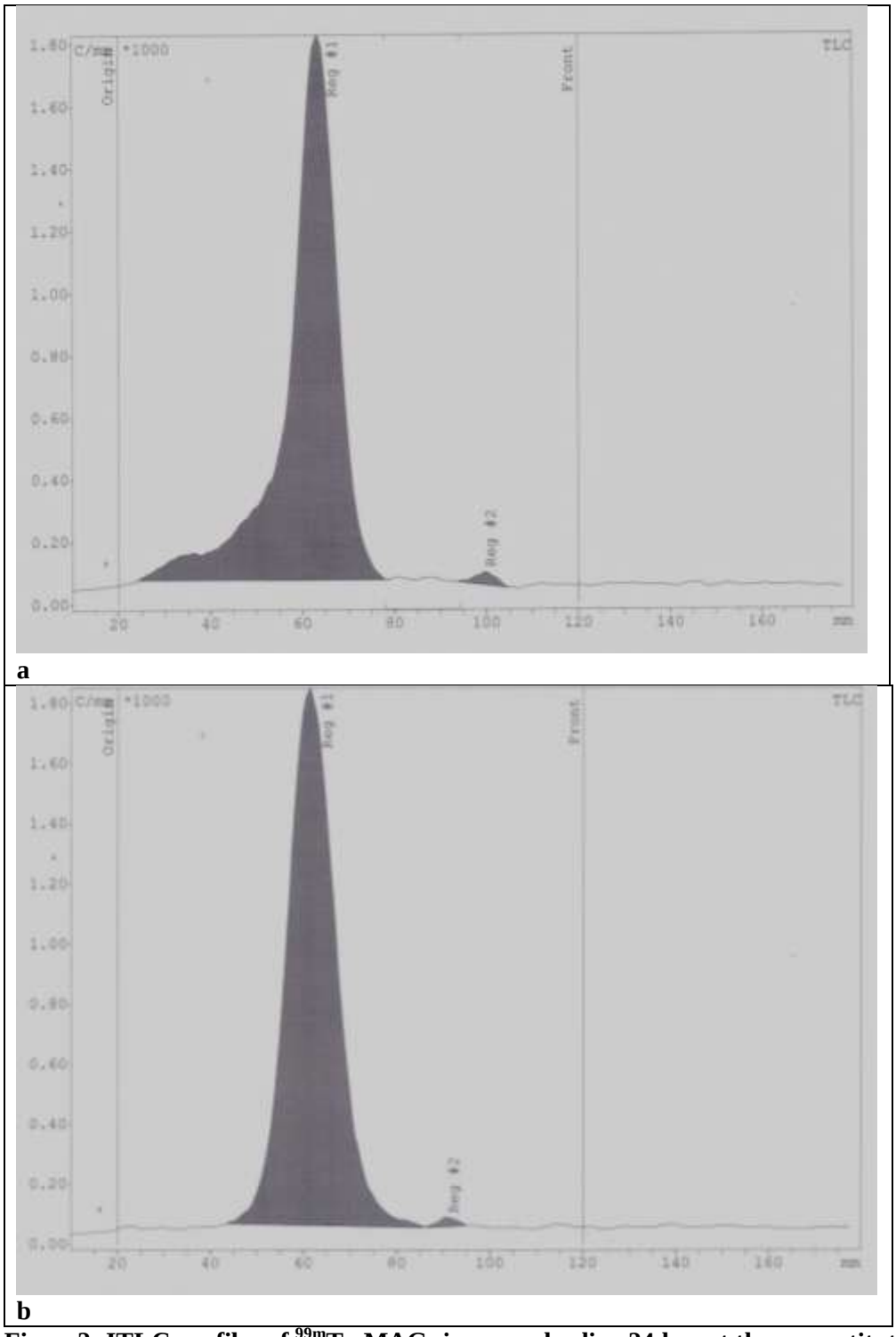


Figure2: ITLC profiles of ^{99m}Tc-MAG_3 in normal saline 24 h post the reconstitution.

Chromatogram on Whatman No:2 of Radiotracer samples by using Acetonitrile60%: normal saline 40% as mobile solvent system. The R_f of $^{99m}TcO_2$, free $^{99m}TcO_4$ and ^{99m}Tc-MAG_3 are 0.00, 0.7-1 and 0.7-1 respectively. The radiotracer samples were prepared by a: Boiling water bath, b: ultrasound radiation.

The Radio- HPLC study was undertaken in order to provide the further information about the efficiency of preparation of ^{99m}Tc-MAG_3 radiotracer samples. The Radio-HPLC assay was performed with analytical

reverse-phase on a JASCO 880-PU intelligent pump HPLC system (Tokyo, Japan) equipped with a multiwavelength 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-MAG$_3$complex by HPLC, a volume of 10µl of the test solution was injected into the C-18 reverse-phase column and trifluoroacetic acid 0.1%/water (solvent A) and acetonitrile (solvent B) were used as a mobile in following gradient: 0 min A 95% (B 5%), 5 min A 95% (B 5%), 25 min A 0% (B 100%) and 30 min A 0% (B 100%), flow= 1 ml/min Figure2.The stability of ^{99m}Tc-MAG$_3$ radiotracer samples were investigated in normal saline up 24 h post the reconstitution at ambient temperature Fig 3.

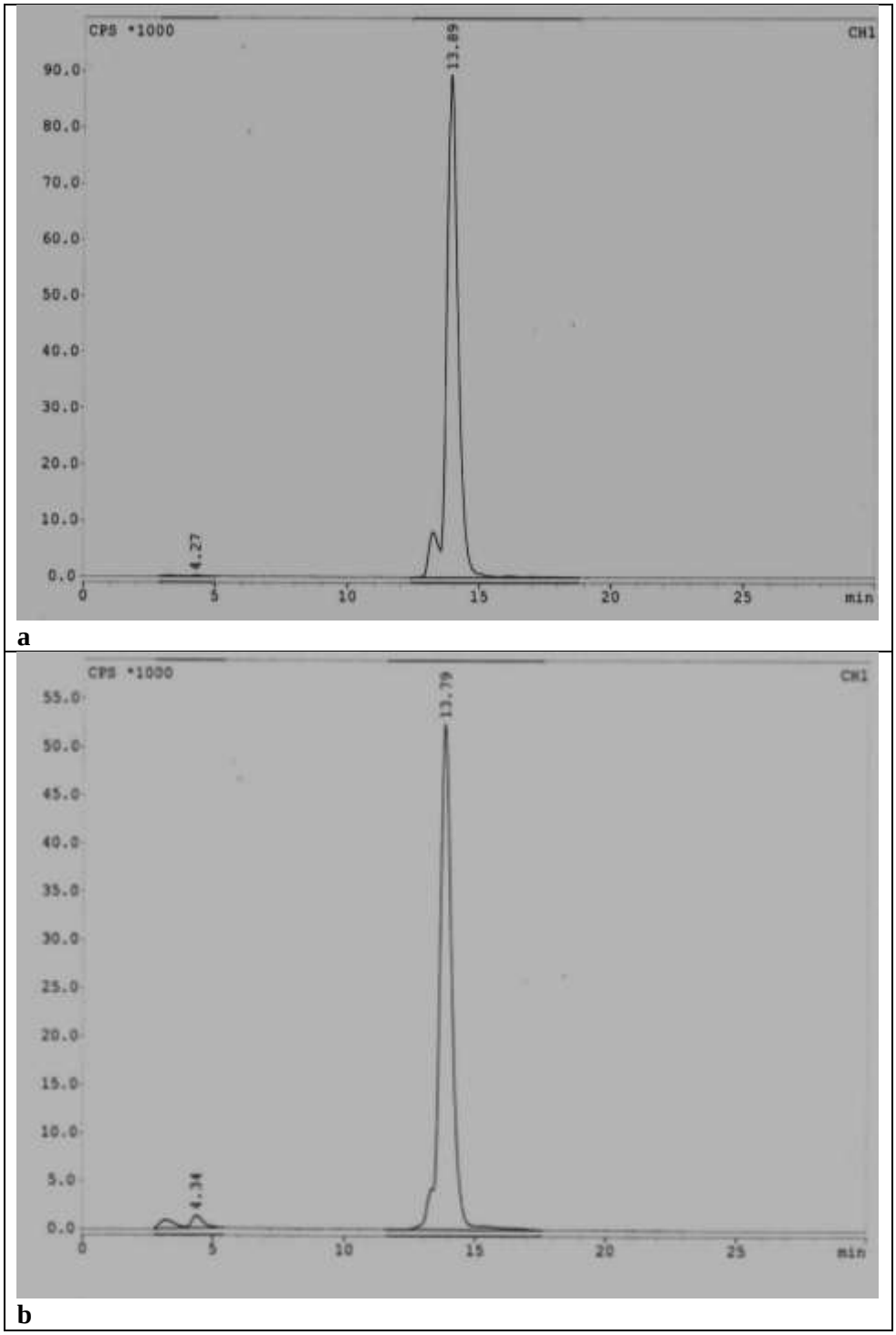


Figure 3: Radio-HPLC chromatogram profiles in normal saline.

The ^{99m}Tc-MAG$_3$ samples were prepared a: Boiling water bath method, b: ultrasound radiatiom. The retention times of free ^{99m}TcO4 and ^{99m}Tc-MAG$_3$ are approximately 4 and 14 min respectively.

Partition coefficient

The partition coefficient is the ratio of the concentration of a chemical compound in a mixture of two miscible hydrophilic and lipophilic solvents at the equilibrium stage. This is an indicator of the difference in the solubility of chemical compounds in these two phases. The distilled water and 1- octanol were used to measure the hydrophilicity of ^{99m}Tc-MAG$_3$ complex samples which were prepared by the boiling water bath or the new developed techniques. 1 ml of distilled water and 1 ml octanol were dispensed in the empty vial, and then 100 µl the radiotracer sample added to the mixture. The vial was vigorously shaken by shaker for 10 min and then centrifuged at 500×g for 5 min at room temperature. Three aliquots of 50 µl were sampled from each layer and counted by the gamma counter. The mean activities from the octanol and water layers were calculated for each radiotracer test sample. The octanol to water partition coefficient (P O/W) was measured by dividing the counts of the octanol phase by that of the aqueous phase. The partition coefficient factor is considered of logarithm P O/W.

Protein bonding

Protein bonding of ^{99m}Tc-MAG$_3$ radiotracer samples was undertaken as the following procedure. 1 ml of freshly human serum albumin (purchased from the Iranian Transfusion Organization Tehran) was added to 100 µl of the final solution of radiotracer sample in micro tube. The mixtures were gently shaken for 10 min and incubated at incubator at 37°C for 1 h. Then each sample was treated by 1 ml of ethanol and centrifuged at 500×g for 10 min at the room temperature and followed by decanting the supernatant from debris. The activity of each portion was quantified by gamma counter. The protein bonding of radiotracer sample or radio-metal transferred to serum albumin was calculated by dividing the activity of precipitated protein to the total activity of sediment and supernatant multiple 100.

Biodistribution of ^{99m}Tc-MAG$_3$ radiotracer in the rat

The rat was placed in the restrainer device and the 37 MBq (1 mCi) ^{99m}Tc-MAG$_3$was injected intravenously by contra lateral tail vein in all studies. The rats were returned back to their cages and kept individually. Radioisotope analysis was performed 1 h post injection. The rats were sacrificed by diethyl ether. The organs of interest such as kidneys, liver, stomach, spleen, intestine, bladder, heart, and lungs were removed. The relative activity of each organ to the interest organs was calculated. The results have been obtained from this analysis, stated in table 2.

Table 2: The relative uptake of ^{99m}Tc-MAG$_3$ samples which were prepared by boiling water bath or sonication methods.

Organs / Uptake	Intestine	kidneys	liver	Bladder	Stomach	Spleen	Lungs	Heart
Boiling	41.18± 1.05	25.62±4.31	12.76±1.48	7.3±3.1	6.22±2.96	3.28±0.44	1.8±0.53	1.84±0.65
Sonication	39.7±5.3	25.54±1.1	12.26±3.3	6.94±1.45	7.5±0.72	3.16±1.1	1.2±0.24	3.6±1.2

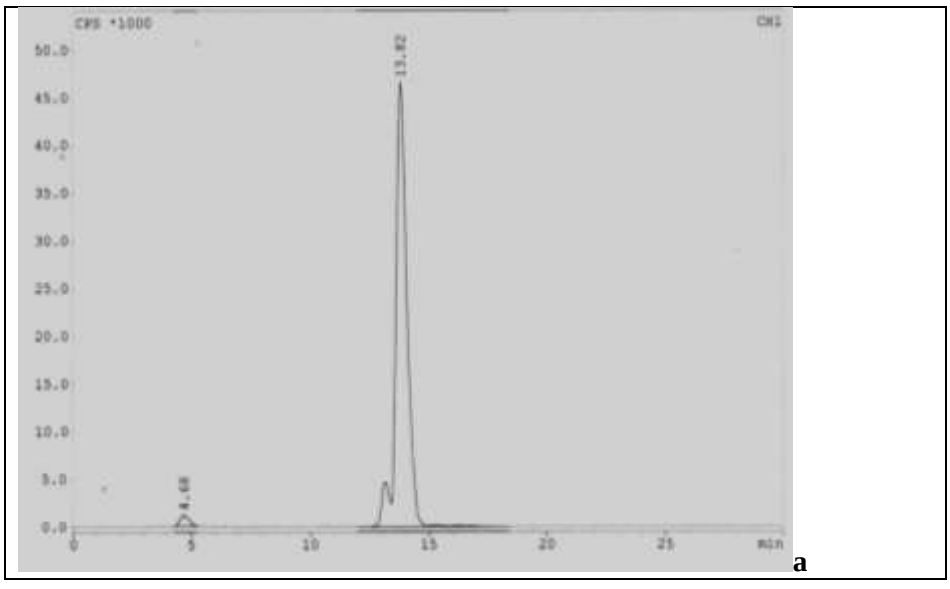
The 37MBq (1 mCi) of radiotracer sample was injected intravenously by contra lateral tail vein. The rats were sacrificed by diethyl ether 1 h post injection. The organs of intestine, kidneys, liver, bladder, stomach, spleen, lungs and heart are removed and counted by gamma counter for 2 min. Relative activity of each organ was measured by dividing the activity of organ to the total activity of interest organs.

Statistical analysis

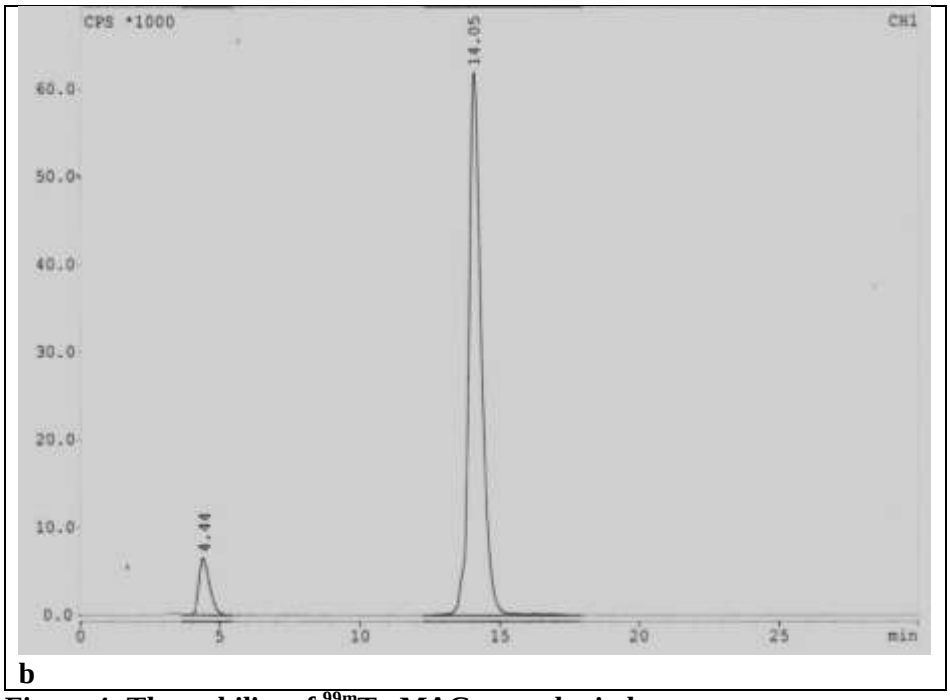
The calculations of means and standard deviations were made on Microsoft Excel. The data were shown as the mean ± SD.

Results

The free $^{99m}TcO_4$ and $^{99m}TcO_2$ are two major radiochemical impurities produced during the radiolabeling of a ligand with ^{99m}Tc radioisotope. The radiotracer complex sample, $^{99m}TcO_4$and $^{99m}TcO_2$ are readily identified and quantified by ITLC analysis. The $^{99m}TcO_2$radiochemical impurity could not be detected by Radio-HPLC assay. Twenty freeze-dried cold kits of MAG_3 were chosen and divided into two groups equally. The first group of vials was reconstituted by boiling water bath as the conventional or standard method according to the manufacturer's instructions. The radiolabeling of second group was undertaken in the ideal condition by ultrasound irradiation, which this condition was determined in the preliminary experiment. The yields (n= 10) of ^{99m}Tc-MAG_3, free $^{99m}TcO_4$ and $^{99m}TcO_2$ were 94.45± 1.1, 3.7± 0.8 and 1.85± 0.65 when the radiolabeling reactions were carried out by boiling water bath method. For the samples (n=10) were reconstituted under ultrasound waves, these values were 92.15± 0.44, 3.65± 0.28 and 4.2±.0.35 respectively. As Radio-ITLC profiles are shown in figure 1 and, the chromatogram characteristics of radiotracer samples in the two different mobile systems were similar for the all radiocomplex samples were prepared by both methods. The Radio-HPLC assay demonstrated that all radiolabeling reactions were leaded to single radio complex samples. The retention times for free $^{99m}TcO_4$ and ^{99m}Tc-MAG_3 radio complex sample were approximately 4and 14 min respectively. The retention time of radio complex samples was identical for all radiotracer samples which they were prepared in this approach. The yields of free ^{99m}TcO4 and ^{99m}Tc-MAG_3 samples were 98.1± 0.8 and 1.9± 0.15 respectively when the radio complex samples were prepared by the standard method in Radio-HPLC analysis. These values were 95.04± 0.65 and 4.96± 0.45 respectively when the radio complex samples were prepared by the new developed technique. The retention time of radio complex samples were prepared by sonication, was similar to the retention time of samples that they were prepared by the standard method. The similarity of retention times was the strong evidence for successful preparation of ^{99m}Tc-MAG_3 through the application of ultrasound waves. The radiopharmaceutical can be injected to the patient must have radiochemical purity above 90 % in accordance to the manufacturer's instructions. All samples had radiochemical purity higher than 90% when they were prepared through sonication under ideal condition. The stability of ^{99m}Tc-MAG_3 samples was examined in the normal saline solution over 24 h after their preparations by ITLC and Radio-HPLC assays. The outcome of this analysis indicated that the radiotracers were stable in the normal saline up 24 h post the reconstitution. The decomposition of the radiotracer samples has not been observed in this period. The partition coefficient factor was -2.4± 0.32 for the^{99m}Tc-MAG_3samples (n= 10)which were prepared by the standard method. The value of this factor was -2.51± 0.42 for the radiotracer samples (n= 10) which were reconstituted by sonication. The negative values of partition coefficient factor indicated the hydrophilic characteristic of radio complex samples which were prepared by two methods and a tendency towards the kidneys. The protein bonding of ^{99m}Tc-MAG_3samples were prepared by boiling water bath (n= 10) or ultrasound irradiation (n= 10) 35.23± 1.15 and 39.36± 1.7 respectively. The significant statistical difference has not been observed in these values between the radio complex samples in which were prepared by either two above mentioned techniques in this investigation.



a



b

Figure 4: The stability of ^{99m}Tc-MAG$_3$ samples in human serum.

Radio-HPLC chromatograms have been obtained from the supernatant solution of ^{99m}Tc-MAG$_3$ samples in human serum. a: Boiling water bath, b: ultrasound radiation.

The stability of radio complex samples was checked in human serum at 37 °C. As it is stated in Fig 4, the radiocomplex samples showed good stability in human serum and the radiochemical purity of radiotracer samples remained above 90% under physiologic condition. The radioisotope analysis was performed in order to investigate the biodistribution of ^{99m}Tc-MAG$_3$samples in the rats. As it is shown in table 2, the radiolabeling procedure could not lead to a significant difference in the biodistribution of radiotracer samples. The highest activity was measured in the intestine followed the kidneys and liver.The radiolabeling procedures have not influenced on the biodistribution pattern of radiocomplex samples particularly in the kidneys. The in-vitro and in-vivo tests were carried out and substantially confirmed that the ^{99m}Tc-MAG$_3$samples prepared by sonication had the identical properties as those radio complex samples prepared by the conventional method.

Discussion

^{99m}Tc radioisotope is widely used for radiolabeling of a variety of different ligands in nuclear medicine in which are suitable for imaging and assessment of various desired organs. Its popularity is related to the suitable emitted gamma energy (140 keV), absence of beta radiation and appropriate physical half-life. The effective half-life of ^{99m}Tc radionuclide ($t_{1/2}$= 6.03 h)is not so short that imaging cannot be done or not too long for patients to be hospitalized for imaging procedure. In addition to the above mentioned factors, it can be supplied to nuclear medicine centers as a generator ^{99}Mo/^{99m}Tc. The^{99m}Tc obtained from the generator in the form of Na^{99m}TcO$_4$ has the highest state of oxidation cannot react with the unshared electrons of ligand. If the ^{99m}Tc radioisotope is used to label ligands for imaging, it should be reduced to a lower oxidation state by a reducing agent present in the formulation of the kits, except in the case where the ^{99m}Tc in the form of Na^{99m}TcO$_4$ is used for imaging. In order to carry out the radiolabeling reaction of the ligand with ^{99m}Tc radioisotope, the ligand structure must contain functional groups that contain non-bonded electrons such as oxygen, nitrogen, sulfur, phosphorus etc, to react with empty orbitals of reduced ^{99m}Tc. It is obvious, the nature of covalent co-ordinate (dative covalent) bonding is not strong like covalent or ionic bonds. The reaction of some ligands with reduced ^{99m}Tc is readily performed at room temperature for radiolabeling reaction[22]. However, in some ligands, the radiolabeling reaction is facilitated by heating due to the fact the functional groups in these ligand structure do not share non-bonding electrons readily with the reduced ^{99m}Tc[19-21,23,24]. Boiling water bath device is routinely used for heating source in nuclear medicine centers. The radiolabeling procedure is usually performed at 100 °C for 10 to 30 min depending on the radiopharmaceutical agents according to the protocols provided by manufacturers. Inadequate heating, caused by insufficient incubation

temperature or insufficient incubation time, may not provide the necessary energy to drive the reaction to completeness and therefore results in unacceptably high amounts of residual, unreacted free $^{99m}TcO_4$. This dilemma has been reported for a variety ^{99m}Tc-Ligands[25-27]. If the radiolabeling reaction is carried out in milder condition or shorter time especially in emergency situations, it can be considered as a significant measure in clinical practice. The use of microwave wavelengths was also proposed for the synthesis of methoxy isobutyl isocyanate (MIBI) and for the preparation of ^{99m}Tc-MIBI (Sestamibi) radiotracer [28]. Despite the fact that radiolabeling reaction of MIBI with ^{99m}Tc was carried out in shorter time in comparison to boiling water bath method, this modality is not commonly used for radiopharmaceutical works due to the following limitations. The geometry of the samples was very important when the samples were placed in the microwave oven instrument. The high potential risk of sparking for the presence of metal cap. Microwave instrument with digital control panel is suitable for setting short heating time, since it must be accurately set at the required heating period. Any technical error in setting the instrument heating time below or beyond the predetermined time may be leaded the reconstitution of radiopharmaceutical kit rendered inappropriate for clinical usage. Any residual gas left in the head space of the vial could cause an ejection of the rubber stopper due to the excess steam built up the vial. In addition to the aforementioned factors, the loss of variation of microwave oven output and frequency related to extended use of the device must be inspected on a long-term usage in order to obtain the radiotracer samples with high efficiency and radiochemical purity[29]. Sonication deals with passage of ultrasound waves to increase or alter chemical reactions. Sound is waves of compression and expansion moving through medium. Therefore, sonication can enhance the reaction rates due to small cavities which implode, creating tremendous heat and pressure, shock waves and particle accelerations [30,31]. The energy released by the passage of sound waves could provide the required energy for the radiolabeling reaction of freeze-dried kit MAG_3 with ^{99m}Tc. The radiochemical purity of ^{99m}Tc-MAG_3 radiotracer samples was prepared with the help of sound waves without heating had no specification properties for imaging in patients. The combination of sonication and heating at 60 °C could provide the required energy in order to produce the radiotracer samples with appropriate yields for imaging. The bonding between ligand and reduced ^{99m}Tc was not strong. Hence, the radiolabeling efficiency was decreased if the radiolabeling reaction was carried out at higher than 60°C. The reaction time was another important factor to produce ^{99m}Tc-MAG_3 samples. The yield of radiolabeling was reduced again when the reaction time was persisted more than 1 min at 60 °C. The decomposition of radio complex could be happened if the reaction time and temperature were increased beyond ideal condition under sonication. The preparation of ^{99m}Tc-MAG_3 radiotracer by using ultrasound waves has the following advantages in comparison to the boiling water bath method. The reaction of MAG_3 with ^{99m}Tcis carried out in milder condition. The reaction time was considerably reduced. The process of reaction is not required to have a special technical skill and is enough to put the lead shield vial inside the ultrasound instrument. It is possible to be used by any nuclear medicine centers. The potential absorbed radiation to the staff are working in the nuclear medicine departments can be decreased. Energy consumption may be reduced and therefore, effective in reducing the cost of preparation radiotracer. Since the temperature and the reaction time are two main factors for the preparation of radiopharmaceutical agent with suitable yield, and the ultrasound instrument has various models and capabilities available on the market. It is necessary that the ideal condition for determining these two factors be performed in order to apply the new developed technique for radiopharmaceutical work. It is mandatory that the legal considerations of using sonication for the preparation of ^{99m}Tc-MAG_3 must be judged and approved by officials for clinical application.

Conclusion

The outcome of our investigation indicated that the ^{99m}Tc-MAG_3 samples were prepared with high efficiency by sonication. The new developed technique was reproducible and reliable to produce radiotracer samples. This method can be recommended as an alternative for the reconstitution of ^{99m}Tc-MAG_3 forrenography study. Green chemistry has been opened a new way to prepare radiotracer for radiopharmaceutical work. This modality can be used for the preparation of any radiotracer samples which the reconstitutions are routinely facilitated by heating due to boiling water bath method.

Acknowledgement

This study is part of Pharm-D thesis of Sepide Ghasemi Falavarjani. The authors have no relevant financial interests related to the material in this manuscript. They also have no conflict of interests to declare. This work has been carried out with financial support (grant No: U-95123)from Ahvaz Jundishapur University

of Medical Sciences, Ahvaz, Iran. The authors gratefully acknowledge boss and staff of Radioisotope Division of Atomic Energy Organization Iran for assistance, good cooperation and the other contributions.

Abbreviations

Bq: Becquerel, Ci: Curie, MAG_3mercaptoacetyltriglycine,Mo: Molybdenum,Tl: Thallium, TcO_4^-: Pertechnetate, Tc: Technetium

References

1. Taylor, A. J. R., Jr., & Nally, J. V. (1995). Clinical applications of Renal Scintigraphy. *AJR. American Journal of Roentgenology*, 164(1), 31–41. doi:10.2214/ajr.164.1.7998566.
2. Boubaker, A., Prior, J. O., Meuwly, J. Y., & Bischof-Delaloye, A. (2006). Radionuclide investigations of the urinary tract in the era of multimodality imaging. *Journal of Nuclear Medicine*, 47, 1819–1836.
3. Salih, S., & Yousef, M. (2013). Wl-WathiqMabrook M, AliOmer MA. Evaluation of 99mTc-MAG3/DTPA in detection of hydronephrosis. *Life Science Journal*, 10, 3522–3527.
4. Hauser, W., Atkins, H. L., Nelson, K. G., & Richards, P. (1970). Technetium99m DTPA: a new radiopharmaceutical for brain and kidney scanning. *Radiology*, 94(3), 679–684. doi:10.1148/94.3.679.
5. Qi, Y., Hu, P., Xie, Y., Wei, K., Jin, M., Ma, G., . . ., & Chen, X. (2016). Glomerular filtration rate measured by (99m) Tc-DTPA renal dynamic imaging is significantly lower than that estimated by the CKD-EPI equation in horseshoe kidney Patients. *Nephrology (Carlton, Vic.)*, 21(6), 499–505. doi:10.1111/nep.12663.
6. Fritzberg, A. R., Kasina, S., Eshima, D., & Johnson, D. L. (1986). Synthesis and biological evaluation of technetium-99m MAG3 as a hippuran replacement. *Journal of Nuclear Medicine*, 27, 111–116.
7. Itoh, K. (2001). 99mTc-MAG3: review of pharmacokinetics, clinical application to renal diseases and quantification of renal function. *Annals of Nuclear Medicine*, 15(3), 179–190. doi:10.1007/BF02987829.
8. Wong, J. C. H., Rossleigh, M. A., & Farnsworth, R. H. (1995). Utility of technetium-99m-MAG3 diuretic renography in the neonatal period. *Journal of Nuclear Medicine*, 36, 2214–2219.
9. Taylor, A. J. R., Jr., Eshima, D., & Alazraki, N. (1987). 99mTc-MAG3, a new renal imaging agent: preliminary results in patients. *European Journal of Nuclear Medicine*, 12(10), 510–514. doi:10.1007/BF00620476.
10. Jafri, R. A., Britton, K. E., Nimmon, C. C., Solanki, K., Al-Nahhas, A., Bomanij, J., . . ., & Hawkins, L. A. (1988). Technetium-99m MAG3, a comparison with iodine-123 and iodine-131 orthoiodohippurate, in patients with renal disorder. *Journal of Nuclear Medicine*, 29, 147–158.
11. Inoue Y, Ohtake T, Yokoyama I, Yoshikawa K, Asai S, Ohtomo K. Evaluation of renal function from 99m Tc-MAG3 without blood samplingJournal of Nuclear Medicine, 1999,40;793-798 .
12. Clausen, T. D., Kanstrup, I. L., & Iversen, J. (2002). Reference values for 99mTc-MAG3renography determined in healthy, potential renal donors. *Clinical Physiology and Functional Imaging*, 22(5), 356–360. doi:10.1046/j.1475-097X.2002.00443.x.
13. Demirel, B. B., Balci, T. A., Tasdemir, B., & Pinar Koc, Z. (2012). Comparison of DTPA and MAG3 renal scintigraphies in terms of differential renal function based on DMSA renal scintigraphy. *Pakistan Journal of Medical Sciences*, 28, 795–799.
14. Strasser, C., Haid, B., Langsteger, W., & Oswald, J. (2016). Benfit of F-15 protocols in equivocal F+D MAG3renography in children with upper tract dilatation and systemic split function: results and outcomes. *Journal of Pediatric Urology*, 12(5), 295e1–295.e6. doi:10.1016/j.jpurol.2016.04.038.
15. Khalaj, A., Doroudi, A., & Adibpour, N., MohseniAraghi G. (2009). N-alkylation and N-acylation of 2,4-dinitrophenylamine by ultrasound. *Asian Journal of Chemistry*, 21, 997–1001.
16. Allahyari, S., Haghighi, M., Ebadi, A., & Hosseinzadeh, S. (2014). Ultrasound assisted co-precipitation of nanostructured CuO-ZnO-Al2O3 over HZSM-5: effect of precursor and irradiation power on nanocatalyst properties and catalytic performance for direct syngas to DME. *Ultrasonics Sonochemistry*, 21(2), 663–673. doi:10.1016/j.ultsonch.2013.09.014.
17. Safaei-Ghomi, J., & Akbarzadeh, Z. (2015). Sonochemically synthesis of arylethynyl linked triarylaminescatalyzied by CuI nanoparticles: a rapid and green procedure for songashira coupling. *Ultrasonics Sonochemistry*, 22, 365–370. doi:10.1016/j.ultsonch.2014.05.016.

18. Mirza-Aghayan, M., Ganjbakhsh, N., Molaee Tavana, M., & Boukherroub, R. (2016). Ultrasound-assisted direct oxidation of benzyl alchohols catalyzed by graphite oxide. *Ultrasonics Sonochemistry*, 32, 37–43. doi:10.1016/j.ultsonch.2016.02.017.
19. Doroudi, A., Saadati, S. M., Pour, H. H., Ahmadi, F., Erfani, M., Rezaee, S., & Etesami, B. (2013). Preparation of 99mTc-MIBI complex under ultrasound irradiation. *Journal of Radioanalytical and Nuclear Chemistry*, 298(2), 1185–1190. doi:10.1007/s10967-013-2559-y.
20. Doroudi, A., Erfani, M., Norouzi, B., Saadati, S. M., Kiasat, A., Ahmadi, F., & Baghersad, M. H. (2015). Clinical application of ultrasound for preparation of 99mTc-sestamibi cpmplex. *Annals of Nuclear Medicine*, 29(3), 295–301. doi:10.1007/s12149-014-0941-7.
21. Doroudi, A., Saraji, F., Erfani, M., Saadati, S., Kisast, A., Ahmadi, F., & Etesami, B. (2016). The stability of 99mTc-MIBI (Sestamibi) complex samples which prepared under ultrasound irradiation technique versus boiling water bath method. *Journal of Applied Pharmaceutical Science*, 6, 126–134. doi:10.7324/JAPS.2016.601120.
22. Doroudi, A., Erfani, M., Moradpour, S. A., Saadati, S. M., Ahmadi, F., Kiasat, A., . . ., & Etesami, B. (2017). The evaluation effect of omeprazole effect on the sensitivity of 99mTc-MDP bone-seeking to detect simulated closed fracture in the rat's foot. *Iranian Journal of Nuclear Medicine*, 25, 92–99.
23. Gandomkar, M., Najafi, R., Shafiei, M., Mazidi, M., Goudarzi, M., Mirfallah, H., . . ., & Abdie, N. (2009). Clinical evaluation of antimicrobial peptide [99mTc/Tricine/HYNIC] Ubiquicidin 29-41 as a human-specific infection imaging agent. *Nuclear Medicine and Biology*, 36(2), 199–205. doi:10.1016/j.nucmedbio.2008.11.003.
24. Erfani, M., & Shafiei, M. (2014). preparation of 99mTc-TRODAT-1 with high labeling yield in boiling water bath: a new formulation. *Nuclear Medicine and Biology*, 41(4), 317–321. doi:10.1016/j.nucmedbio.2014.01.003.
25. Haney, T. A., Ascanio, I., Gigliotti, J. A., Gusmano, E. A., & Bruno, G. A. (1971). Physiacal and biological properties of a 99m Tc-sulfur colloid preparation containing disodium edetate. *Journal of Nuclear Medicine*, 12, 64–68.
26. Gagnon, A., Taillefer, R., Bavaria, G., & Leveille, J. (1991). Fast labeling of technetium- 99m-sestamibi with microwave oven heating. *Journal of Nuclear Medicine Technology*, 19, 90–93.
27. Eshima, D., Eshima, L. A., Gotti, N. M., Herda, S. C., Algozine, C. A., Burris, T. G., . . ., & Taylor, A. T. (1996). Technetium-99m-sulfur colloid for lymphoscintigraphy: effects of preparation parameters. *Journal of Nuclear Medicine*, 37, 1575–1578.
28. Lima, M. J. C., Marques, F. L. N., Okamoto, M. R. Y., Garcez, A. T., Sapienza, M. T., & Buchpiguel, C. A. (2005). Preparation and evaluation of modified composition for lyophilized of [Cu(MIBI)4]BF4 for [99mTc] technetium labeling. *Brazilian Archives of Biology and Technology*, 48, 1–8. doi:10.1590/S1516-89132005000700001.
29. Hung, J. C., Wilson, M. E., & Gibbons, R. J. (1991). Rapid preparation and quality control method for technetium-99m-2-methoxy isobutyl isonitrile (technetium-99m-sestamibi). *Journal of Nuclear Medicine*, 32, 162–168.
30. Suslick, K. S. (1990). Sonochemistry. *Nature*, 247, 1439–1445.
31. Ting-Nien, W., & Meng-Chun, S. (2010). pH-affecting sonochemical formation of hydroxyl radicals under 20 kHz ultrasound irradiation. *Sustainable Environmental Research*, 20, 245–250.

***** *****