IJPSR (2019), Volume 10, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 December 2018; received in revised form, 18 March 2019; accepted, 28 March 2019; published 01 September 2019

THE APPLICATION OF ULTRASOUND IRRADIATION AS AN ALTERNATIVE METHOD FOR THE PREPARATION OF ^{99m}Tc-OCTREOTIDE RADIO COMPLEX

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Keywords:

Somatostatin, Sonication, ^{99m}Tc-Octreotide$_3$, Ultrasound irradiation

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ABSTRACT: This approach was launched to evaluate the application of ultrasound waves for the preparation of ^{99m}Tc-Octreotide radio conjugate sample. The 740MBq (20 mCi) of ^{99m}Tc-Octreotide samples were prepared by the boiling water bath (standard) or ultrasound irradiation methods. The stability of samples in normal saline and human serum albumin was investigated. The partition coefficient and protein bonding of samples were determined. The radioisotope analysis was undertaken to assess the biodistribution of radiotracer samples in rats. The data obtained from this approach revealed that the ^{99m}Tc-Octreotide samples were successfully prepared by the newly developed technique. Statistically significant differences have not been observed in the stability, partition coefficient and protein bonding of radiotracer samples which were prepared by either of the two above-mentioned. The radioisotope analysis showed that the applied method for radiolabeling did not lead to a significant difference in the biodistribution pattern of radiotracer samples in rats. Green chemistry can be recommended for the radiolabeling of Octreotide with ^{99m}Tc as an alternative and reliable modality for the boiling water bath method.

INTRODUCTION: Somatostatin is a hormone with 14- amino acid residues. Its biological half-life is about 2 min, and it has an inhibitory effect on some biological processes. The expression of somatostatin receptors with high affinity toward this molecule in some specific tumors and its ability to label with radioisotopes has led to the use of the radiolabeled protein for imaging or treatment of some kinds of tumors in nuclear medicine [1-5].

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DOI: 10.13040/IJPSR.0975-8232.10(9).4176-85

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(9).4176-85

However, due to the short half-life of the radiolabeled peptide molecule, it is not possible to use it as a radiopharmaceutical agent in clinical practice. Hence, various analogs have been developed that have a longer half-life and are more potent and effective than somatostatin. These analogs include Octreotide, Vapreotide and lanreotide [6-9]. Each analog has the potentially affinity to a specific subset of somatostatin receptors and has the capability to react with a suitable radioisotope for the imaging or treatment of special tumors in nuclear medicine. Somatostatin receptor imaging has a pivotal role in the discrimination of pancreatic endocrine tumors, carcinoids, paragangliomas, some central nervous system tumors and small cell or non-small cell lung cancers.

Technetium- 99m octreotide (^{99m}Tc-Octreotide) has been developed as a radiopharmaceutical agent in this regard [10-12]. This radiotracer has the combination of advantages of somatostatin analog and radiolabeling of the ^{99m}Tc radionuclide. ^{99m}Tc-Octreotide is established for the identification of tumors overexpressing somatostatin receptors. The radiolabeling reaction of octreotide with ^{99m}Tc radionuclide is facilitated by heating at 100 °C for 10-15 min according to the manufacturer's instructions. It is highly desirable that the radio-labeling reaction is carried out at the milder condition and shorter time. The radiotracer can be prepared in the sufficient yield and radiochemical purity by official specific requirements for radiopharmaceutical work.

Sonochemistry is the branch of chemistry which deals with the passage of waves to increase or alter chemical reactions [13-16]. Ultrasound waves have chemical effects on the reaction system due to the creation of cavitation or generation of free radicals which augment the rate of reaction. Green chemistry has been recommended for the preparation of technetium- 99m methoxy isobutyl isocyanate (^{99m}Tc-MIBI) radiotracer samples in nuclear medicine [17-19]. The principal aim of this investigation was to evaluate the efficacy of ultrasound irradiation technique to reconstitute the freeze-dried Octreotidekits with ^{99m}Tc radionuclide in comparison to the boiling water bath standard method.

MATERIALS AND METHODS: All commercially available chemicals were procured from Sigma-Aldrich or Merck companies. The solvents were the highest purity and analytical grade and used without further purification. The freeze-dried Octreotide kits and ^{99}Mo/^{99m}Tc generators were provided by the 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' weight 130 ± 15 g was obtained from the research center and the experimental animal house of Ahvaz Jundishapur University of Medical Sciences.

Animal Study: This approach was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences with the code IR, AJUMS, REC, 1396-660. In this investigation, all the guidelines specified by Ahvaz University of Medical Sciences were followed to conduct research on animals. Ten adult, male NMRI rats, were used for this approach. The animals were acclimated to the experiment condition for one week.

The subjects were kept in individual cages in an air-conditioned room at 24 ± 1 ºC with a 12 h light-dark cycle and were fed with a standard pellet diet and had free access to water. The rats were randomly divided into two groups. The radiotracer samples were prepared by the newly developed technique administrated to one group. The radiotracer samples were prepared by the boiling water bath or standard method administrated to the other group. The radio complex samples were administrated intravenously into the rats. The volume of radiotracer was 0.2 ml that was injected into the tail vein of the animals.

The Preparation of ^{99m}Tc-Octreotide and Quality Control: Each lyophilized, freeze-dried kit contains sterile and pyrogen free mixture of hydrazino nicotinamide (HYNIC)-Tyrosine-Octreotide 20 µg, stannous chloride dihydrate 40 µg, tricine 15 µg, and ethylene diamine N, N′ diacetic acid (EDDA) 5 mg under vacuum. The radiolabeling reaction of the ligand with ^{99m}Tc and quality control were carried out according to the manufacturer's specifications. Thus, 0.5 ml of 0.9% saline was added to an evacuated vial and allowed the mixture to preincubate for 5 min. The sterile and non-pyrogenic freshly eluted 740MBq (20mCi) $^{99m}TcO_4^-$ in 0.5 ml saline was aseptically added to the mixture mentioned above. The lead shield vial was slowly shaken for 1 min and the vials placed vials in the boiling water bath for 15 min as the standard method or sonicated in the thermostated bath (Elma, P = 95 w made in Germany) as the newly developed technique.

The quality control was undertaken by Instant Thin Layer Chromatography (ITLC) for the preliminary study. ITLC on Whatman no. 2 as stationary phase performed using two different solvent systems. When acetone (pH = 5) as mobile phase was used the free technetate $^{99m}TcO_4^-$ moved to solvent front (R_f = 0.7-1) and ^{99m}Tc-Octreotide and colloid technetium ($^{99m}TcO_2$) remained at point of

application (R_f = 0.00). When the mixture of water and acetonitrile in the ratio of 1:1 was used as another mobile phase the $^{99m}TcO_2$ remained at the spotting point and free $^{99m}TcO_4^-$ and desired radio complex moved to the solvent front (R_f = 0.7-1), The strips were cut into equal pieces and radioactivity of each part quantified by the propose collimator an energy peak centered a 140 keV with NaI(Tl) detector for 2 min. The radiolabeling yield was calculated by 100- (% $^{99m}TcO_2$ + %$^{99m}TcO_4^-$). The radiolabeling reaction of ^{99m}Tc with ligand was carried out due to sonication at different times and temperature points to determine the standard condition for the reconstitution of radiotracer with the appropriate radiolabeling yield. The number of radiochemical impurities and radiopharmaceutical purity should be within the limits that are determined by the Atomic Energy Organization of Iran. All Iranian nuclear medicine departments must comply with these specific regulations for the preparation of ^{99m}Tc-Octreotide radiotracer by the boiling water method. The radiolabeling yield must be higher than 90% for imaging with^{99m}Tc-Octreotide radiotracer. The radiolabeling reactions were carried out at different temperatures and times to produce the^{99m}Tc-Octreotide radiotracer samples with a radiochemical purity higher than 90% by ultrasound method. All experiments were undertaken three times independently, and the results are shown in **Table 1**.

TABLE 1: THE 740MBq (20 mCi) FRESHLY ELUTED SOLUTION OF $^{99m}TcO_4^-$ WAS ADDED TO FREEZE-DRIED OCTREOTIDE KITS

Temperature °C	Time (min)	$^{99m}TcO_4^-$%	$^{99m}TcO_2^-$%	^{99m}Tc-Octreotide %
	1	36.97 ± 0.49	10.27 ± 0.15	52.76 ± 0.35
	2	32.93 ± 0.72	10.57 ± 0.42	56.5 ± 0.3
30	3	28.18 ± 0.62	12.72 ± 0.43	59.1 ± 1.05
	4	25.02 ± 0.7	17.08 ± 1.8	57.9 ± 1.12
	5	23.27 ± 1.15	16.75 ± 0.62	59.98 ± 1.8
	1	23.59 ± 0.41	22.75 ± 0.06	53.66 ± 0.47
	2	27.64 ± 0.6	13.01 ± 0.02	59.35 ± 0.63
40	3	27.27 ± 4.22	13.31 ± 1.6	59.42 ± 3.6
	4	22.69 ± 0.32	14.9 ± 0.14	62.41 ± 0.18
	5	20.68 ± 12	14.22 ± 0.47	65.1 ± 0.6
	1	25.8 ± 0.21	16.35 ± 0.35	57.85 ± 0.4
	2	18.78 ± 0.74	17.82 ± 0.65	63.4 ± 1.4
50	3	25.1 ± 0.14	11.2 ± 0.42	63.7 ± 0.28
	4	12.9 ± 1.2	21.25 ± 1.76	65.85 ± 3.04
	5	11.15 ± 1.06	21.75 ± 0.78	67.1 ± 0.28
	1	22.85 ± 0.2	19 ± 0.5	58.15 ± 0.1
	2	19.4 ± 5.56	16.66 ± 4.01	63.94 ± 1.5
60	3	17.15 ± 1.06	18.7 ± 1.27	64.15 ± 0.21
	4	14.8 ± 0.2	19.17 ± 0.1	66.03 ± 0.1
	5	12.65 ± 1.4	19.48 ± 1.7	67.87 ± 2.2
	1	21.43 ± 0.74	1.99 ± 0.42	76.58 ± 1.16
	2	10.87 ± 0.3	4.96 ± 0.33	84.17 ± 0.64
70	3	5.57 ± 1.8	6.8 ± 2.25	87.63 ± 2.1
	4	2.37 ± 0.85	6.77 ± 1.02	90.86 ± 0.82
	5	2.5 ± 0.98	3.98 ± 0.7	93.52 ± 0.69
	1	14.4 ± 2.85	14.32 ± 2.1	71.28 ± 2.4
	2	9.2 ± 0.75	13.12 ± 0.84	77.68 ± 1.2
80	3	8.1 ± 1.22	14.8 ± 1.67	77.1 ± 1.4
	4	7.37 ± 2.3	13.5 ± 2.8	79.13 ± 2.46
	5	6.1 ± 1.14	14.36 ± 1.05	79.54 ± 0.9

The vials were sonicated in the thermostated bath (Elma, P= 50 W, Germany) at different temperature and reaction times. Each experiment was repeated three times and yields of free $^{99m}TcO_4^-$, $^{99m}TcO_2$, and ^{99m}Tc-Octreotide species respectively.

When the radiolabeling reaction was carried out at 70 °C for 5 min, the radio conjugate purity was higher than 90% And enhancing the temperature caused the yield to be less than 90 %. Therefore, all the radio complex samples were prepared by the ultrasound irradiation technique in the determined condition for the evaluation of stability radio complex samples and the measurement of partition coefficient and protein bonding factors.

The 20 commercial Octreotide kits were chosen from different batches randomly and divided into

two equal groups. The radiolabeling reaction was undertaken with ^{99m}Tc radioisotope by the standard method in the first group. The radiolabeling of the other group was carried out by the newly developed technique. The ITLC analysis used for a preliminary experiment to determine the optimal condition from the aspect of reaction time and temperature to prepare the radio conjugate with efficient yield. The Radio-HPLC analysis was performed with analytical reverse-phase on a JASCO 880-PU intelligent pump HPLC system (Tokyo, Japan) equipped with a multi-wavelength 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-Octreotide samples 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 the 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 ITLC and radio high performance liquid chromatography (Radio-HPLC) methods used for the evaluation of stability of radio complex samples **Fig. 1** and **2**.

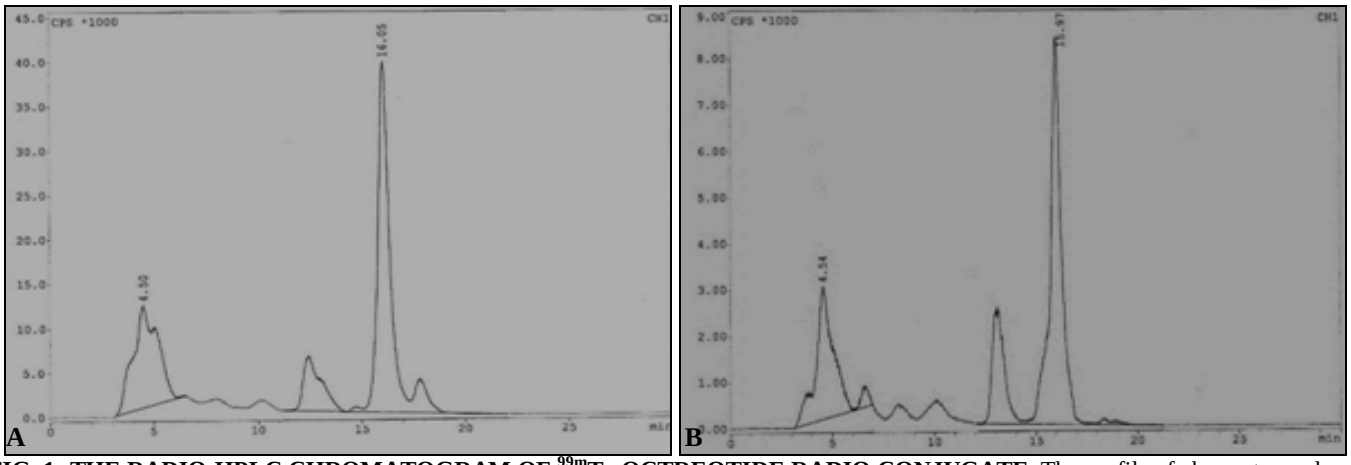

FIG. 1: THE RADIO-HPLC CHROMATOGRAM OF ^{99m}Tc-OCTREOTIDE RADIO CONJUGATE. The profile of chromatographs of radiotracer samples was prepared 1 h post the reconstitution of cold kits due to a: boiling water bath b: ultrasound irradiation methods. The retention times of free $^{99m}TcO_4^-$ and ^{99m}Tc-Octreotide radio complex are approximately 4 and 16 min respectively.

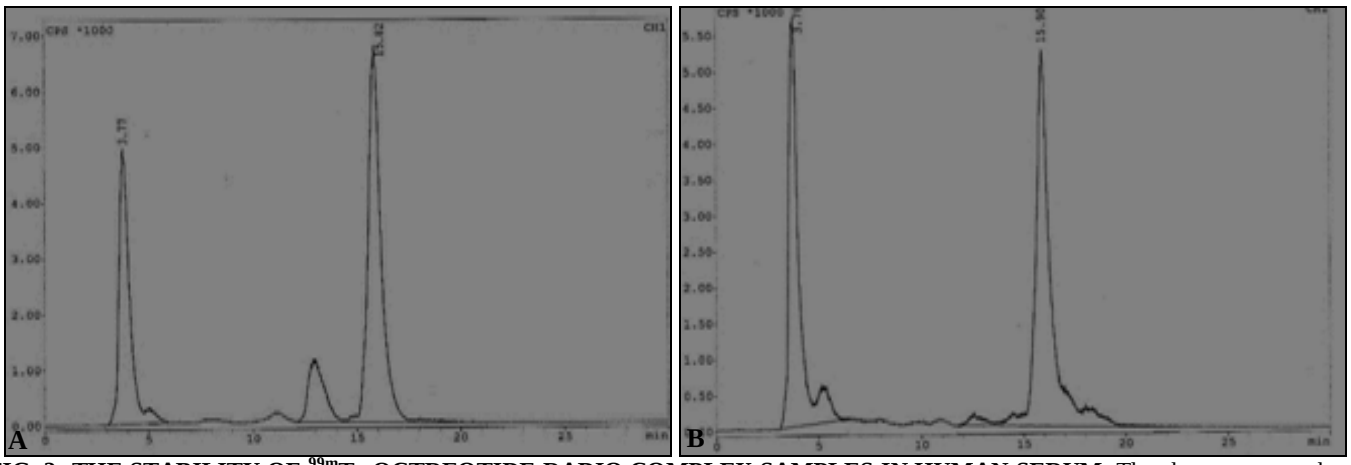

FIG. 2: THE STABILITY OF ^{99m}Tc-OCTREOTIDE RADIO COMPLEX SAMPLES IN HUMAN SERUM. The chromatograms have been obtained from the supernatant solution of radiotracer samples in human serum. The radio conjugate samples were reconstituted by a: boiling water bath b: ultrasound irradiation techniques.

Partition coefficient: Two-phase solvent was used to measure the partition coefficient of radio complex samples which were reconstituted by either two above mentioned methods. Therefore, the 100 µl of prepared radio complex sample was added to the mixture of 1 ml of octanol and 1 ml water in the vial. The mixture was shaken for 5 min and kept at room temperature for 15 min. Then the organic and aqueous phases were separated by centrifugation at 500 × g for 10 min, and the activity of each phase was quantified by a well-type gamma counter. Three aliquots of 50 µl were

sampled from each layer and counted and mean activities from the organic and aqueous phases were calculated for each sample tube. The value of log P was calculated by dividing the activity of the octanol phase by that of the aqueous phase.

Protein Bonding: The measurement of protein bonding of radio conjugate samples was carried out by the following procedure. The amount of 100 µl of radiotracer sample was added to 1 ml of freshly human serum albumin (purchased from the Iranian Transfusion Organization Tehran). The mixtures were gently shaken for 15 min and incubated at 37 °C for 24 h. Then each sample was treated by 1 ml of ethanol and centrifuged at 500 × g for 10 min. The supernatant and debris were separated, and the activity of each part measured by a well-type gamma counter. The protein bonding of radiotracer or radiometal transferred to serum albumin was calculated by the activity of precipitate to the total activity of sediment and supernatant multiple by 100. Aliquots were analyzed with Radio-HPLC to assess the serum stability of a radio complex sample **Fig. 2**.

Biodistribution Investigation: The biodistribution study of ^{99m}Tc-Octreotide radiotracer was performed in the rat model. Each animal was placed in the restrainer device, and the 37 MBq (1 mi) radio conjugated sample administrated intravenously via the contra lateral tail vein in all studies. The rats were returned to their cages and kept individually. The subjects were sacrificed by diethyl ether 1 h post injection. The organs of interest such as kidneys, liver, stomach, spleen, intestine, bladder, heart, and lungs, right foot, left foot, and pancreas was removed, and activity of each organ was counted by a well-type gamma camera. The relative activity of each organ to the interest organs was calculated. The results obtained from this analysis are stated in **Table 2**.

TABLE 2: THE BIODISTRIBUTION ANALYSIS OF ^{99m}Tc-OCTREOTIDE SAMPLES WHICH WERE PREPARED BY THE BOILING WATER BATH OR SONICATION METHODS

Organ	Boiling Water Bath Uptake %	Ultrasound Irradiation Uptake %
Intestine	42.86 ± 5.52	40.57 ± 1.08
Kidneys	20.72 ± 2.84	24.85 ± 1.4
Lungs	1.48 ± 0.22	1.04 ± 0.05
Bladder	2.46 ± 1.90	10.38 ± 2.5
Stomach	2.23 ± 0.34	1.91 ± 0.05
Spleen	0.19 ± 0.03	0.28 ± 0.02
Right foot	10.27 ± 4.7	6.8 ± 0.1
Left foot	11.49 ± 4.51	7.42 ± 0.7
Pancreas	3.13 ± 0.45	2.73 ± 0.07
Heart	0.18 ± 0.03	0.28 ± 0.02
Liver	4.99 ± 0.48	3.74 ± 0.15

The 37MBq (1 mCi) radio conjugate was injected intravenously to the rats. The animals were sacrificed by diethyl ether 1 h post injection. The organs of Intestine, Kidneys, Lungs, Bladder, Stomach, Spleen, Right foot, Left foot, Pancreas, Heart, and Liver were 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 mean and standard deviation (SD) values were performed using Microsoft Excel software. All data were expressed as the mean ± SD. Repeated measure design analysis of variance followed by the Tukey test was used to assess the difference between the biodistribution of radiotracer samples that administrated into two groups of rats. Statistical significance was considered at $p<0.05$.

RESULTS: The chromatography technique is widely used for the quality control of analysis in nuclear medicine. Free $^{99m}TcO_4^-$, $^{99m}TcO_2$, and radioligand can be readily identified and quantified by ITLC, but $^{99m}TcO_2$ is not detected by Radio-HPLC analysis. A total of twenty freeze-dried vials of Octreotide were selected from different batches. The vials were randomly divided into two groups equally. The radiolabeling of one group was carried out by the boiling water bath using the manufacturer's instructions.

The reconstitution other group was undertaken by the newly developed method at the ideal condition that was obtained in the preliminary investigation. When the radiotracer samples (n=10) were prepared by the conventional method, the percent of free $^{99m}TcO_4$, $^{99m}TcO_2$ and $^{-99m}$Tc-Octreotide

radio complex were 1.5 ± 0.6, 2.1± 0.45 and 96.5 ± 1.15 respectively. The percent of free $^{99m}TcO_4^-$ and desired radio conjugate sample were 2.65 ± 0.55 and 97.35 ± 0.45 respectively by Radio-HPLC analysis. These values were 2.1 ± 0.6, 4.75 ± 0.9 and 93.15 ± 0.67 by ITLC and 3.72 ± 0.41 and 96.28 ± by Radio-HPLC investigations respectively when the radio complex samples were prepared by the ultrasound irradiation technique. The yield of radiopurity was above 90% in all experiments. As shown in **Fig. 1** and **2**, the retention times of $^{99m}TcO_4^-$ and ^{99m}Tc-Octreotide were approximately 4 and 16 min respectively when the radio complex samples were prepared by the boiling water bath or ultrasound irradiation methods. The Radio-HPLC analysis indicated that the values of retention times for free $^{99m}TcO_4^-$ and ^{99m}Tc-Octreotide radio complex were identical. Therefore, the radio conjugate samples were successfully prepared by the newly developed technique. The stability analysis of ^{99m}Tc-Octreotidesamples was investigated in the normal saline solution up to 24 h post reconstitution in which either of the two above-mentioned methods prepared the samples. As shown in **Fig. 3** and **4**, the radio conjugate samples were completely stable, and the disintegration of radiotracer samples was not observed during this period. The partition coefficient factor was -2.052 ± 0.26 for the ^{99m}Tc-Octreotide samples (n= 10) which were prepared by the boiling water bath method. This value was -2.106 ± 0.18 for the samples (n = 10) which were reconstituted by the new developed technique.

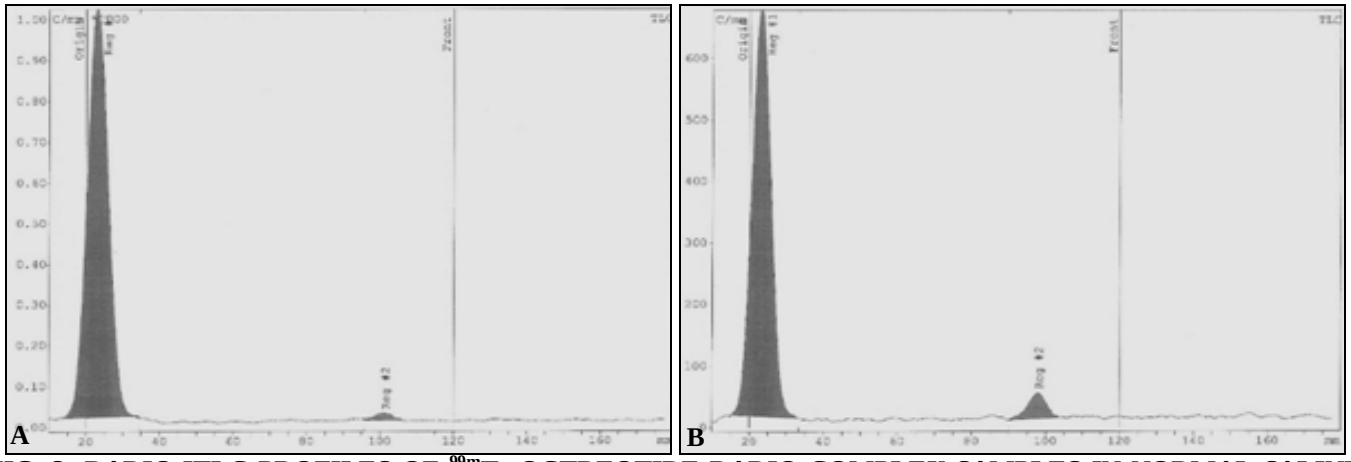

FIG. 3: RADIO-ITLC PROFILES OF ^{99m}Tc-OCTREOTIDE RADIO COMPLEX SAMPLES IN NORMAL SALINE OVER 24 h THE PREPARATION. The mobile phase and stationary phases were acetone and paper Whatman no. 2. The R_f of $^{99m}TcO_2$, ^{99m}Tc-Octreotide and free $^{99m}TcO_4^-$ are 0.00, 0.00 and 0.7-1 respectively. The radio complex samples were prepared by a: boiling water bath, b: ultrasound irradiation methods

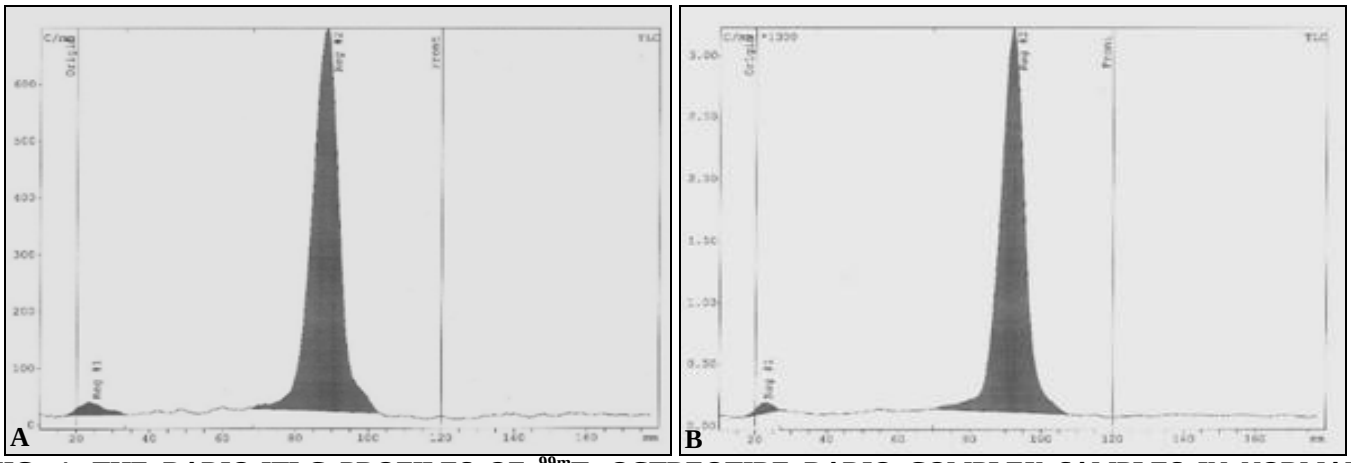

FIG. 4: THE RADIO-ITLC PROFILES OF ^{99m}Tc-OCTREOTIDE RADIO COMPLEX SAMPLES IN NORMAL SALINE OVER 24 h POST THE PREPARATION. The solvent system was a mixture of water and acetonitrile (50:50%). The chromatograms obtained on Whatman no. 2 as the stationary phase. The R_f of $^{99m}TcO_2$, free $^{99m}TcO_4^-$ and ^{99m}Tc-Octreotide were 0.00, 0.7-1 and 0.7-1 respectively. The radiotracer samples were prepared by a: boiling water bath, b: ultrasound irradiation methods.

The negative value of this factor revealed this matter that the radiotracer has the hydrophilic property and tendency towards to excrete via the kidneys. The protein bonding of the ^{99m}Tc-Octreotide samples was prepared by the boiling water bath (n= 10) or sonication (n= 10) 62.65 ± 2.4 and 66.91 ± 2.25 respectively. The stability of the radio conjugate samples was assessed in human serum at 37 °C. As shown in **Fig. 4**, the radio complex samples showed good stability in human serum, and the radiochemical purity of the radiotracer samples remained above 90% under physiologic condition.

The biodistribution of the radio conjugate samples was performed to evaluate the distribution of the ^{99m}Tc-Octreotidesamples in rats. As shown in **Table 2**, the radiolabeling procedures did not lead to a significant difference in the pattern of biodistribution of the radiotracer samples. The highest activity was measured in the intestine followed by the kidneys. Result indicated that the method of the preparation of ^{99m}Tc-Octreotidesamples could not lead to altering the properties of radiotracer samples in the bioanalysis study. The outcome of all *in-vitro* and *in-vivo* investigations demonstrated that the characteristics of ^{99m}Tc-Octreotidesamples were reconstituted by the newly developed technique was completely similar to the properties of the radiotracer samples were prepared by the standard method.

DISCUSSION: A radiopharmaceutical agent is a chemical compound containing a radioactive ingredient in its structure. Most radiopharmaceutical agents are used in nuclear medicine for diagnostic procedures. They are usually pharmacologically inactive due to the tiny amount of radiotracer is administrated to humans. It is the most important factor for the difference between radiopharmaceutical and pharmaceutical agents. The radiochemical purity of a radiotracer is the fraction of the total radioactivity in the desired chemical form. The radiochemical impurities have a variety reason including disintegration due to the action of the solvent, change in temperature or pH, exposure to light, the presence of oxidizing or reducing agents in the formulation of a kit, incomplete reaction and radiolysis [20]. The presence of radiochemical impurities during the radiolabeling reaction can reduced the quality of imaging due to a decrease in the target to non-target ratio. ^{99m}Tc radionuclide is the most intensive radioisotope used for the radiolabeling of ligands for imaging purposes in nuclear medicine departments. Its popularity is related to the suitable half-life ($t_{½}$ = 6.03 h), ideal photon gamma radiation (140keV) and absence of beta radiation. It can be readily supplied as a ^{99m}Mo/^{99m}Tc generator for nuclear medicine departments particularly for those centers which are far away from nuclear reactors [21]. The effective half-life of ^{99m}Tc is not so short that imaging cannot be done or not too long for patients to be hospitalized for the imaging procedure.

In addition to the factors mentioned above, a variety of ligands have unshared electron groups such oxygen, nitrogen, sulfur and phosphorous which can be reacted with the reduced form of the ^{99m}Tc radionuclide. Therefore, a different assessment of the structure and function of the organs is carried out by radiopharmaceutical agents that are labeled with ^{99m}Tc in clinical practice. ^{99m}Tc is obtained from a ^{99}Mo/^{99m}Tc generator in the form of pertechnetate (^{99m}TcO4$^-$). ^{99m}Tc in the $^{99m}TcO_4^-$form has the highest oxidation state, and the radiolabeling reaction of ligands cannot be performed. For this reason, it is necessary that the $^{99}mTcO_4^-$ in the eluted solution must be reduced to the lower oxidation state in order for radiolabeling reactions are carried out for radiopharmaceutical works. 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 $^{99m}TcO_4^-$ is used for scintigraphy imaging.

Therefore, most ^{99m}Tc- ligands except $^{99m}TcO_4^-$ itself, are reconstituted by the reduction of $^{99m}TcO_4^-$ in the presence of a ligand agent. The bonding between reduced ^{99m}Tc and ligand is covalent co-ordinate (dative covalent). It is very obvious, the nature of this kind of bonding versus the covalent or ionic bonds is not strong. The radiolabeling reaction of some ligands with reduced ^{99m}Tc is readily carried out at room temperature like diethylene triamine pent acetic acid (^{99m}Tc-DTPA) and methylene diphosphonate (^{99m}Tc-MDP) [22, 23]. However, in some ligands, the radiolabeling reaction is facilitated by heating due to the fact that

the functional groups in this ligand structure do not share non-bonding electrons readily with the reduced ^{99m}Tc like ^{99m}Tc-TRODAT-1 [24]. Heating with the boiling water bath device is commonly used for the preparation of radiopharmaceutical kits. The heating with the technique mentioned above is usually time-consuming.

The radiolabeling reactions could potentially suffer from the following precaution factors. 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 $^{99m}TcO_4$ and produced an unacceptable amount of 99mTcO_2. This dilemma has been reported for a variety of ^{99m}Tc-Ligand samples to the literature [25-27]. The gas may build up the sealed vial due to overheating. The gas pressure is enough to cause the rupture of the septum of the vial. Then the cap of the vial can be ejected, or the glass walls broken. This factor is very important for spilling the content of the vial and contamination of the environment or staff who are working in the nuclear medicine departments. It is highly desirable for radiopharmacy work, the reliable modality versus the boiling water bath method can be developed for the preparation of radiotracer samples in a shorter time or milder condition, the reconstitution of Sestamibi radiotracer was suggested by micro oven as an alternative method.

The ^{99m}Tc-Sestamibi could be prepared with sufficient yield due to the new developed technique. The radiolabeling reaction time was reduced to 10 seconds. 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 practically used for the preparation of ^{99m}Tc-Sestamibiradiotracer due to the following precautions. The geometry of the vials is one of the most important factors when they were put inside the device. The risk of sparking might be enhanced by the presence of a metal cap. The digital control panel of a microwave instrument is usually for setting short heating time. Therefore, the time of required heating must be set accurately. If any technical error happened in setting the instrument heating time below or beyond the predetermined time, the radiolabeling reaction may cause the radiotracer rendered inappropriate for imaging. Any residual gas left in the headspace of the vial could cause ejection of the rubber stopper due to the excess steam built up in the vial.

In addition to the factors above, 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 to obtain the radiotracer samples with high efficiency and radiochemical purity. Despite the significant reduction in the time of radiolabeling process in comparison to the boiling water bath method, the suggested method is not commonly used for the preparation of radiotracer in nuclear medicine due to the limitations of using this technique [28]. The chemical reactions can be carried out due to the ultrasound irradiation technique. This method has recently been taken into consideration by a variety of researchers for chemical reactions. Passing sound waves from the solution can be an important factor in enhancing or changing the pathway for the chemical reactions. The speed of reaction can be effectively increased by ultrasound waves due to small cavities which are created in the solution[29-30].

The energy released by the passage of sound waves could supply the required energy for the reactions. The radiolabeling reaction could take between the reduced form of ^{99m}Tc and Octreotide by sonication. But the radiopurity was lower than the specific requirement for radioisotope imaging. The combination of ultrasound and heating at 70 °C could provide the required energy to prepare the radio complex samples with appropriate yields which were suitable for imaging. The bonding between the ligand and reduced ^{99m}Tc was not strong. Hence, the radiolabeling efficiency was decreased if the radiolabeling reaction was carried out at higher than 70 °C. The reaction time was another important factor to produce ^{99m}Tc-Octreotide samples. The preparation of ^{99m}Tc-Octreotide radio complex has the following advantages when ultrasound irradiation technique is used.

The radio labeling reaction is carried out under milder condition. The reaction time is decreased in comparison to the boiling water bath method. The process of radiolabeling reaction is not required to

have special technical skill. It is enough to put the lead shield vial inside the thermostated bath of the sonication instrument. It is possible to be used by any nuclear medicine centers. Because the radiolabeling reaction is performed at the milder condition by sonication versus the boiling water bath method, the staff is responsible for the preparation of the radiotracer can be effectively received less ionization irradiation. The cost expenditure of radiotracer's preparation can be reduced by saving energy consumption.

The outcome of this investigation demonstrated that the temperature and reaction time are two important factors for the preparation of ^{99m}Tc-Octreotide radio complex with high radiochemical purity by the newly developed technique. The radiochemical purity and impurities are in the range specified by the kits manufacture. A variety of sonication devices with different powers are available in the market. Therefore, the preparation of ^{99m}Tc-Octreotide radio complex using this method, is necessary to determine the optimum condition for obtaining the radio conjugate sample with appropriate radiochemical purity. Then, the newly developed technique can be applied for the reconstitution of the ^{99m}Tc-Octreotide radiotracer. The legal considerations must be approved by officials for the application of sonication for the preparation of ^{99m}Tc-Octreotide radio conjugate in nuclear medicine.

CONCLUSION: The results of this study indicated that sonication could be recommended for the preparation of ^{99m}Tc-Octreotide radio complex sample. Green chemistry is an alternative method the radiolabeling of Octreotide cold kit with the ^{99m}Tc radionuclide. The suggested method is completely accountable and reliable. Sonication can effectively open a new pathway in radiopharmacy work. It can be applied for the reconstitution of any cold kits with ^{99m}Tc radioisotope which the preparations are routinely facilitated by heating due to the boiling water bath method.

ACKNOWLEDGEMENT: This study is part of Pharm-D thesis of Mahshid Hemmati. The project was financially supported by the Research and Technology Deputy of Ahvaz Jundishapur University of Medical Sciences with grant number: U-96113. The authors wish to thank all staffs of Radioisotope Division of Atomic Energy Organization Iran for their help and recommendations on this work.

CONFLICT OF INTEREST: The authors have no relevant financial interests related to the material in this manuscript. They also have no conflict of interests to declare.

REFERENCES:

1. Bison SM, Konijnenberg MW, Melis M, Pool SE, Bernsen MR, Teunissen JJ, Kwekkeboom DJ and de Jong M: Peptide receptor radionuclide therapy using radiolabeled somatostatin analogs: focus on future developments. Clin transl Imaging 2014; 2(1): 55-66.
2. Nedrow JR, White AG, Modi J, Nguyen K, Chang AJ and Anderson CJ: Positron emission tomographic imaging of copper 64 and gallium 68-labeled chelator conjugates of the somatostatin agonist try3-octrotide. Mol Ima 2014: 13.
3. Graf F, Fahrer J, Maus S, Morgenstern A, Bruchertseifer F, Venkatachalam S, Fottner C, Weber MM, Huelsenbeck J, Schreckenberger M, Kaina B and Miederer M: DNA double-strand breaks as a predictor of efficacy of the alpha-particle emitter Ac-225 and the electron emitter Lu-177 for somatostatin receptor targeted radiotherapy. PLos One 2014; 9(2): e88239.
4. Dash A, Chakraborty S, Pillai MR and Knapp FF: Peptide receptor radionuclide therapy: an overview. Cancer Biother Radiopharm 2015; 30(2): 47-71.
5. Puszkiel A, Bauriaud-Mallet M, Bourgeois R, Dierickx L, Courbon F and Chatelut E: Evaluation of the interaction of amino acid infusion on 177Lu-dotatate pharmacokinetics in patients with gastroenteropancreatic neuroendocrine tumors. Clin Pharmacokinet 2019; 58(2): 213-22. doi: 10.1007/s40262-018-0674-1.
6. Grimberg A: Somatostatin and cancer: applying endocrinology to oncology. Cancer Biol Ther 2004; 3(8): 731-33.
7. Wang Z, Ma W, Wang J, Deng J, Yuan M, Zhao M, Jia L, Yang W, Zhang R, Qin W, Chu D and Li G: Imaging and therapy of hSSTR2-transfected tumors using radiolabeled somatostatin analogs. Tumour Biol 2014; 13(4): 2451-57.
8. Ito T, Honma Y, Hijioka S, Kudo A, Fukutomi A, Nozaki A, Kimura Y, Motoi F, Isayama H, Komoto I, Hisamatsu S, Nakajima A and Shimatsu A: phase II study of lanreotideautogel in Japanese patients with unresectable or metastatic well-differentiated neuroendocrine tumors. Invest New Drugs 2017; 35(4): 499-08.
9. Aalbersberg EA, de Wit-Van der Veen BJ, Versleijen MWJ, Saveur LJ, Valk GD, Tesselaar MET and Stokkel MPM: Influence of lanreotide on uptake of 68Ga-DOTATATE in patients with neuroendocrine tumours: a prospective intra-patient evaluation. Eur J Nucl Med Mol Imaging 2019; 46(3): 696-703. doi: 10.1007/s00259-018-4117-x.
10. Pirayesh E, Amoui M and Assadi M: Uptake difference by somatostatin receptors in a patient with neuroendocrine tumor: ^{99m}Tc-Octrrotide uptake in the lung without uptake in liver lesions. Mol Imaging Radionucl Ther 2015; 24(3): 128-31.
11. Rodrigues NR, Calich AL, Etchebehere M, Ichiki WA, Pereira FP, Amstalden EM and de SA Etchebehere EC.

Whole-body (99m) Tc-Octreotide scintigraphy with SPECT/CT to detect occult tumor-inducing paraneoplastic osteomalacia. Clin Nucl Med 2015; 40(1): 54-57.
12. Serrano-Vicente J, Infante-Torre JR, Garcia-Bernardo L, Moreno-Caballero M, Martizez-Esteve A and Rayo-Madrid JI: Pancreatic accessory spleen: false positive with 99mTc-Octrotide. Rev Esp Med Nucl Imagen Mol 2017; 36(1): 53-55.
13. Khalaj A, Doroudi A, Adibpour N and MohseniAraghi G: N-alkylation and N-acylation of 2,4-dinitrophenylamine by ultrasound. Asia J Chem 2009; 21(2): 997-01.
14. Allahyari S, Haghighi M, Ebadi A and Hosseinzadeh S: Ultrasound-assisted co-precipitation of nanostructured CuO-ZnO-Al_2O_3 over HZSM-5: effect of precursor and irradiation power on nanocatalyst properties and catalytic performance for direct syngas to DME. Ultrason Sonochem 2014; 21(2): 663-73.
15. Safaei-Ghomi J and Akbarzadeh Z: Sonochemically synthesis of arylethynyl linked triarylaminescatalyzied by CuI nanoparticles: a rapid and green procedure for songashira coupling. Ultrason Sonochem 2015; 22: 365-70.
16. Mirza-Aghayan M, Ganjbakhsh N, Molaee-Tavana M and Boukherroub R: Ultrasound-assisted direct oxidation of benzyl alcohols catalyzed by graphite oxide. Ultrason Sonochem 2016; 32: 37-43.
17. Doroudi A, Saadati SM, Hassanpour H, Ahmadi F, Erfani M, Rezaee S, Khodayar MJ, Samaee H and Etesami B: Preparation of ^{99m}Tc-MIBI complex under ultrasound irradiation. J Radioanal Nucl Chem 2013; 298: 1185-90.
18. Doroudi A, Erfani M, Norouzi B, Saadati SM, Kiasat A, Ahmadi F, Etesami B and Baghersad MH: Clinical application of ultrasound for preparation of ^{99m}Tc-sestamibi complex. Ann Nucl Med 2015; 29(3): 295-01.
19. Doroudi A, Saraji F, Erfani M, Saadati SM, Kiasat A, Ahmadi F and Etesami B: The stability of ^{99m}Tc-MIBI (Sestamibi) complex samples which prepared under ultrasound irradiation technique versus boiling water bath method. J Appl Pharma Sci 2016; 6(11): 126-34.
20. Ha NS, Sadeghi S and van Dam RM: Recent progress toward microfluidic quality control testing of radiopharmaceuticals. Micromachines 2017; 8(11): 331.
21. Monroy-Guzman F, Jimenez Martinez TS, Arriola H and Gandara LC: Magnesium-molybate compounds as matrix for 99Mo/99mTc generators. Pharmaceuticals 2011; 4(2): 215-32.
22. Chun-Chen L, Bing-Fu S, Shin-Lin S and Jeng-Daw T: Potential role of Tc-99m DTPA diuretic renal scan in the diagnosis of calyceal diverticulum in children. Medicine 2015; 94(24): e985.
23. Doroudi A, Erfani M, Moradpour SA, Saadati SM, Ahmadi F, Kiasat A, Khodayar MJ and Etesami B: The evaluation effect of omeprazole effect on the sensitivity of ^{99m}Tc-MDP bone-seeking to detect simulated closed fracture in the rat's foot. Iran J Nucl Med 2017; 25(2): 92-99.
24. Saadati SM, Kiasat A, Mousavinia M, Ahmadi F, Ahadi R, Rafie S, Etesami B, Erfani B and Doroudi A: Clinical evaluation of new formulation of ^{99m}Tc-TRODAT-1 for SPECT in Parkinson patients and volunteer subjects. Int J Pharm Res Sci 2018; 9(8): 1000-10.
25. Haney TA, Ascanio I, Gigliotti JA, Gusmano EA and Bruno GA: Physical and biological properties of a ^{99m}Tc-sulfur colloid preparation containing disodium edetate. J Nucl Med 1971; 12(2): 64-68.
26. Eshima D, Eshima LA, Gotti NM, Herda SC, Algozine CA, Burris TG, Vansant JP, Alazraki NP and Taylor AT: Technetium-99m0sulfur colloid for lymphoscintigraphy: effects of preparation parameters. J Nucl Med 1996; 37(9): 1575-78.
27. Gagnon A, Taillefer R, Babaria G and Leveille J: Fast labeling of technetium- 99m-sestamibi with microwave oven heating. J Nucl Med Technol 1991; 19(2): 90-93.
28. Hung JC, Wilson ME and Gibbons RJ: Rapid preparation and quality control method for technetium-99m-2-methoxy isobutyl isonitrile (technetium-99m-sestamibi). J Nucl Med 1991; 32(11): 162-68.
29. Lim M, Son Y and Khim J: The effects of hydrogen peroxide on the sonochemical degradation of phenol and bisphenol A. Ultrason Sonochem 2014; 21(6): 1976-1981.
30. Soumia F and Petrier C: Effect of potassium monopersulfate (oxone) and operating parameters on sonochemical degradation of cationic dye in an aqueous solution. Ultrason Sonochem 2016; 32: 343-47.

How to cite this article:
Doroudi A, Hemmati M, Saadati SM, Kiasat A, Ahmadi F, Etesami B, Khodayar MJ and Erfani M: The application of ultrasound irradiation as an alternative method forthe preparation of ^{99m}Tc-Octreotide radio complex. Int J Pharm Sci & Res 2019; 10(9): 4176-85. doi: 10.13040/IJPSR.0975-8232.10(9).4176-85.