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RESEARCH ARTICLE

PREPARATION OF ^{99M}TC-UBI $_{29-41}$ COMPLEX SAMPLES UNDER ULTRASOUND IRRADIATION TECHNIQUE

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ABSTRACT

This study was launched to evaluate the efficacy of ultrasound irradiation technique versus boiling water bath method for preparation of 99mTc-UBI 29-41 samples and assess the bio distribution of radiotracer samples in the infected rats induced by Staphylococcus aureus. The 740MBq (20 mCi) 99mTc-UBI 29-41 samples were reconstituted by boiling water bath or ultrasound irradiation technique. The 99mTc-UBI 29-41 samples were administrated intravenously to the infected rats. Then qualitative and quantitative studies have been performed. The ITLC and radio-HPLC studies have demonstrated the 99mTc-UBI 29-41 samples with good yields could be prepared by new developed technique. All affected foot's rats could be visualized by scintigraphy imaging. The imaging studies have indicated that the radiotracer samples prepared due to the above mentioned modalities shows very identical bio distribution in the infected rats. The ultrasound irradiation technique is convenient and efficient method to reconstitute the 99mTc-UBI 29-41 kits. It can be recommended as alternative method to prepare infection-seeking radiotracer samples in radioisotope imaging in the clinical practice.

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INTRODUCTION

Ubiquicidin $(UBI)_{29-41}$ is a cationic , synthetic peptide molecule with molecular weight 1.69 KDa (Melendez-Alafort *et al*., 2003; Ferro-Flores et al., 2004). The amino acid sequence of this peptide is Thr-Gly-Arg-Ala-Lys-Arg-Arg-Met-Gln-Tyr-Arg-Arg. It has six positively charged residues. UBI $_{29-41}$ can bind to the negative charge groups present on the microbial membrane by electrostatic attraction forces (Welling *et al*., 2002; Ferro-Flores and Murphy *et al*., 2003) . Therefore, UBI $_{29-41}$ peptide molecule can be localized at the infection foci. This synthetic antimicrobial peptide molecule has potential capability of labeling by ^{99m}Tc radioisotope which widely used in nuclear medicine for diagnosing scintigraphy imaging procedures. UBI $_{29-41}$ is suitable candidate to label by ^{99m}Tc radioisotope directly (^{99m}Tc-UBI $_{29-41}$) or by using coligand reagents like 6-hydrazinopyridine 3-carboxylic acid (HYNIC) and tricine [^{99m}Tc/Tricine/HYNIC]UBI $_{29-41}$ Fig. 1, indirectly for visualizing the infection foci by radioisotope scintigraphy imaging (Gandomkar and Najafi and Mazidi *et al*., 2009; Gandomkar and Najafi andShafiei *et al*., 2009). The ^{99m}Tc-UBI $_{29-41}$ radiopharmaceutical agent has been demonstrated rapid accumulation in the infection regions and fast clearance with minimum liver uptake and hepatobiliary excretion (Melendez-Alafort *et al*., 2004; Akhtar *et al*., 2005). Therefore, ^{99m}Tc-UBI $_{29-41}$ radiopharmaceutical kits have been established as an infection- seeking radiotracer in order to tag at the septic foci. The labeling UBI $_{29-41}$ kit by ^{99m}Tc radioisotope is performed according to the instructions provided by the manufacture. It is usually added freshly eluted ^{99m}Tc as sodium pertechnetate aseptically to the UBI $_{29-41}$ kit. The shielded vial is placed in a boiling water bath at 100ºC for

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10 min. After heating, the shielded vial is placed at room temperature for 15 min. The conventional method for reconstitution of ^{99m}Tc-UBI $_{29-41}$ radiopharmaceutical complex is time-consuming. Ultrasound irradiation technique has widely used in chemistry (Khalaj *et al*., 2006; Khalaj *et al*., 2009; Khorshidi and Tabatabaeian, 2011; Singh *et al*., 2011; Du and Li, 2012). This new developed technique is more convenient and carried out in higher yields, shorter time or milder conditions under ultrasound irradiation technique. In the previous investigation, we could successfully demonstrate that the 37MBq (1 mCi) technetium 99m 2-methoxy isobutyl isonitirle (^{99m}Tc-MIBI, is called Sestamibi) radiopharmaceutical complex samples could be prepared under ultrasound irradiation method with the appropriate yields. The biodistribution of ^{99m}Tc-MIBI complex samples which were prepared by ultrasound irradiation technique investigated in the rat's heart (Doroudi *et al*., 2013). Then the new developed technique has been introduced as an alternative method for reconstitution ^{99m}Tc-MIBI complex samples in clinical practice (Doroudi and Erfani *et al*., 2015). We have made decision to continue our approach for the other radiopharmaceutical kit which the reconstitution is time-consuming in nuclear medicine departments. The main aim of this investigation is to prepare the UBI $_{29-41}$ complexes under ultrasound irradiation technique and investigate the bio distribution of samples in the infected rats in comparison to the samples which are prepared by boiling water bath method.

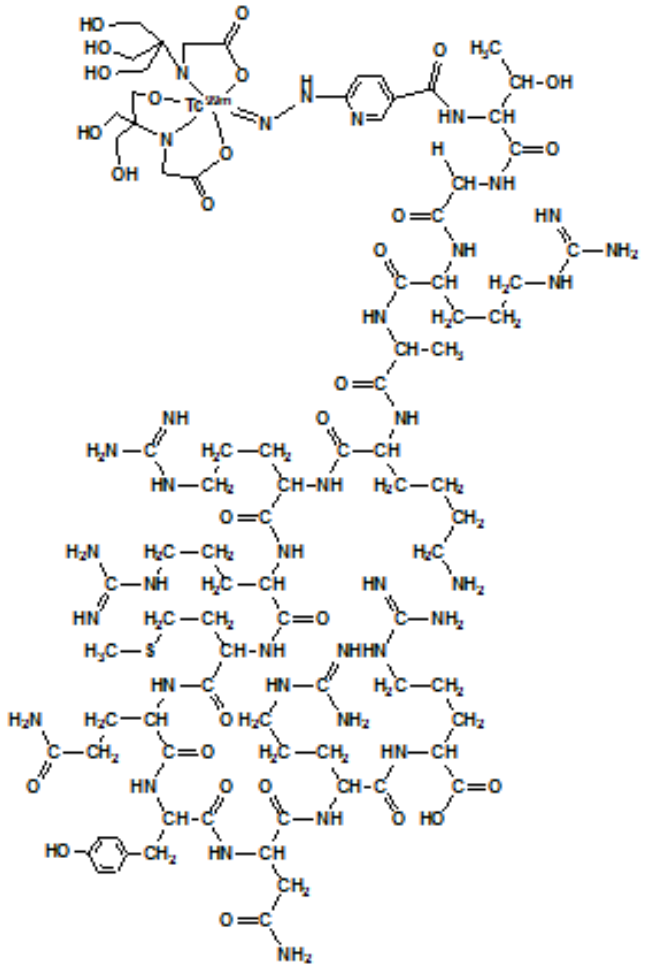


Fig. 1. The structure of [^{99m}Tc/Tricine/HYNIC] UBI $_{29-41}$

MATERIALS AND METHODS

All chemical materials have been purchased from Merck, Fluka and Sigma .The chemicals and solvents were of the highest purity and analytical grade and used without further purification. The freeze-dried kits of [Tricine/HYNIC] UBI $_{29-4}$ and ^{99}Mo/^{99m}Tc generators have been provided by Radioisotope Division of Atomic Energy Organization of Iran (AEOI). The rats with 140±15 g were obtained from research center and experimental animal house of Ahvaz Jundishapur University of Medical Sciences.

Labeling procedure

Commercial [Tricine/HYNIC] UBI $_{29-4}$ kits (AEOI, Tehran, Iran) were used and the labeling was performed according to manufacturer's instructions as follow: 0.5 ml of saline was added to an evacuated vial and shaked, the mixture was allowed to preincubate for 5 min at room temperature. Technetium-99m as sodium pertechnetate was obtained from an in-house ^{99}Mo/^{99m}Tc generator. The 740MBq (20 mCi) freshly eluted $^{99m}TcO_4^-$ in 1 ml saline was added to UBI $_{29-4}$ vials. The vials were heated on the boiling water bath during 10 min, according to manufacturer's instructions as a standard method, or the vials were sonicated at the different temperatures in the thermo stated bath (Elma, p=95W, made in Germany) for 1,2,3,4 and 5 min. Radiochemical analysis studies have been performed by ascending instant thin layer chromatography (ITLC) and radio- High Performance Liquid Chromatography (radio-HPLC).

ITLC analysis was performed for preliminary study not only in order to find out the optimum conditions for the preparation of complex samples with suitable yield under ultrasound irradiation technique, but also in order to avoid consuming huge amounts of solvents with analytical grade which were used by radio-HPLC analysis. ITLC study was performed by using silica gel 60 (Merck) filter paper chromatography as the stationary or the solid phase and different mobile systems. 2-Butanone for free $^{99m}TcO_4^-$ (R_f= 1), 0.1 M sodium citrate pH 5 to determine the nonpeptide-bound ^{99m}Tc coligand with $^{99m}TcO_4^-$ (R_f= 1) and methanol/ 1M ammonium acetate ratio 1:1 for ^{99m}Tc colloid (R_f= 0), R_f values of [^{99m}Tc/Tricine/HYNIC]UBI $_{29-41}$ in each above mentioned system equal 0.0,0.0 and 0.7-1 respectively.

Standard 10 cm in length and 2 cm in width strips were used for each chromatogram. The strips were cut ⅓ lower and ⅔ upper and counted (Aktivimeter, Siemens, Germany) for 2 min under a single head gamma camera equipped with a low energy all-propose collimator .Using an energy peak centered a 140keV. Each experiment was repeated three times and the yields of desired radiotracer calculated. When the radiotracer samples with suitable yield have been reconstituted under new developed technique in comparison to the complex samples which reconstituted due to the boiling water bath method, the HPLC analysis was performed. The quality control of radiotracer samples was performed with analytical reverse phase radio-HPLC on a JASCO 880-PU intelligent pump HPLC system (Tokyo, Japan) equipped with a multi wave 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. The radionuclide analysis of radiotracer complex by radio-HPLC , a volume of 10µl of the test solutions was injected into the C-18 reverse –phase column and o.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 Fig2.

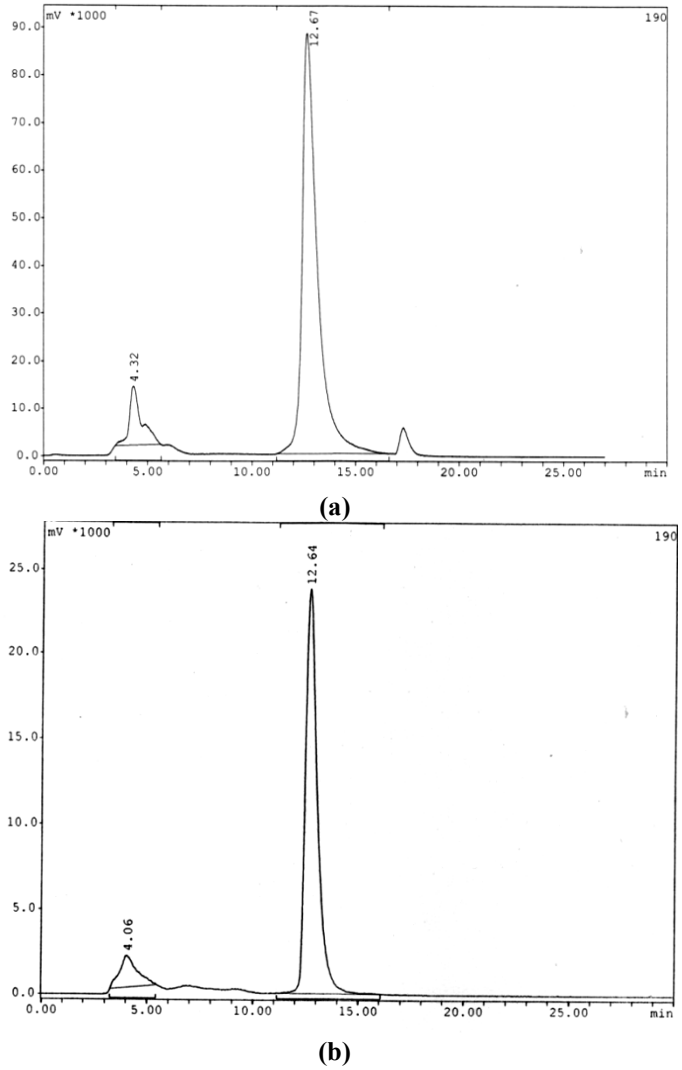


Fig. 2. The radio-HPLC chromatograms of samples were prepared by A: Boiling water bath method B: Ultrasound irradiation method 30 min post reconstitution. The retention time of $^{99m}TcO_4^-$ and radiotracer was approximately 4.06 and 12.64 min respectively

Bacteria Sample

The sample wound swabs were taken from patients admitted to the infection department of Imam Khomeini general hospital. The specimens were transported in sterile, leak-proof container to the microbiology department. The isolates were inoculated on blood agar and incubated overnight at 35 ºC aerobically. Gram- positive cocci occurring in pairs, short chains or clusters, that they were Catalase-positive. Coagulase-positive by test tube and DNase- positive on agar were identified as S aureus and selected.

By using a sterile-tip applicator, touch the surface of one to four morphological identical, isolates colonies. Immerse the applicator into a tube containing Mueller Hinton broth. Rub the tube and mix the cells using a vortex to form a suspension, being careful not to form froth or bubbles in the suspension when mixing the cells. The broth was incubated at 35 ºC, and then the turbidity was adjusted to a number 0.5 McFarland standard. A sterile cotton swab on a wooden stick was dipped into the broth. Excess inoculums were removed rotating the swab against of the tube above the fluid level wall. The Muller-Hinton agar plates were streaked in three dimensions. The plates were inoculated at 35 ºC for 24 hours. The turbidity was adjusted to a number o.5 McFarland (each milliliter of 0.5 McFarland contains 1.5×10^8 microorganisms). Half milliliter of inoculums has been injected to each foot's rat. To make sure about the survival of bacteria, 0.1 milliliter of the above mentioned inoculums was inoculated on blood agar. The experiment has been repeated three times.

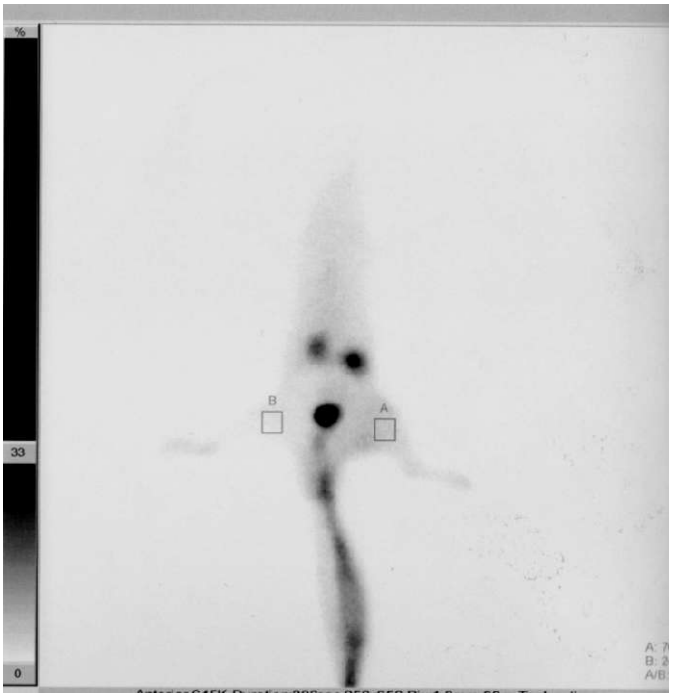
Animal Study

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 20 adult, male NMRI were acclimated to the conditions for one week before the experiment. The animals were kept in individually wire-bottom stainless steel 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 prepared by boiling water bath method administrated to one group and the radiotracer samples were prepared due to ultrasound irradiation method administrated to the other group. Each main group was divided into two subgroups equally. One subgroup was allocated for radioisotope imaging and the other subgroup allocated for quantitative investigation. Each subgroup contained 5 animals. S aureus sample was inoculated into each left foot's rat .The injured area was irrigated with normal saline. The rats were return back to their cages. Imaging and quantitative studies have been performed 48 hours after inoculation of bacteria. The [^{99m}Tc/Tricine/HYNIC] UBI $_{29-41}$ samples were reconstituted by the boiling water or ultrasound irradiation techniques administered to each main group. The rat was placed in the restrainer apparatus and the 37MBq (1 mCi) radiotracer was injected by the contra lateral tail vein.

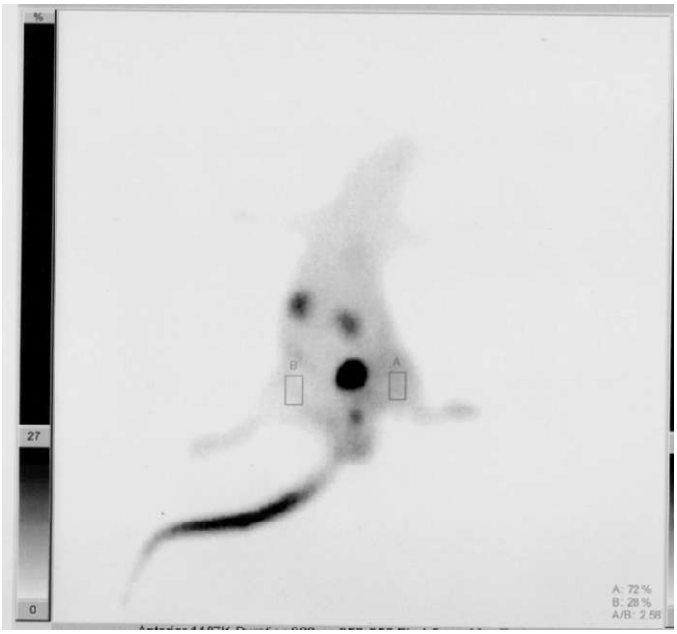
Imaging

The anesthetized live rat was placed in a prone position with limbs spread out and fixed on the board with surgical tape imaging. For all studies a single-headed camera (E-Cam, Siemens, USA) was used. The radioisotope imaging assessment was performed one hour after injection of radiotracer sample. Acquisition parameters were as follows: matrix size 256×256, Zoom factor ×3, anterior and posterior views for 5 min and energy window 140keVand reconstitution Method: filter back projection. Anterior and posterior static images were acquired using a large field of view gamma

camera peaked to 140 Kev with a 15%window and a low-energy all-purpose collimator for 500 kilocounts per image. The gamma camera was positioned to image the affected part and contralateral healthy side.



(a)



(b)

Fig.3. The scintigraphy imaging study has been performed after the 37mBq (1 mCi) radiotracer injected intravenously. The interior view image visualizes the infection lesion induced by S aureus in the left foot's rat. The samples were reconstituted by A: boiling water bath method B: ultrasound irradiation technique

Two criteria have been chosen for interpretation of radioisotope scintigraphy images. First, the visual inspection of the radiotracer uptake at the infected foot to the contralateral healthy side was considered.

Second, by dividing counts in affected foot to the contra lateral healthy side the ratio of affected to unaffected foot (target/non-target ratio) was measured for each subject. Available commercial soft ware was used to quantify the counts. Therefore, region of interest (ROI) was created on the affected foot and then second ROI was generated on the contra lateral unaffected foot in interior views. The specific uptake of radiotracer was calculated by dividing the count per pixel in infected foot to count per pixel in uninfected foot. The background subtraction was not used Fig 3. All scintigraphy images were interpreted by three nuclear physicians independently and their final opinion was achieved by consensus. The observers were unaware of the labeling procedure of radiotracer samples.

Quantitative analysis

The quantitative analysis was performed approximately 1 hour after the infection-seeking radiotracer injected intravenously. The rats were sacrificed by diethyl ether. The organs of interest (infected foot, uninfected foot, lungs, kidneys, liver, bladder, gut, stomach, spleen) removed and weighted. The relative activity factor of each mentioned organs to the interest organs was calculated and shown in Fig 4.

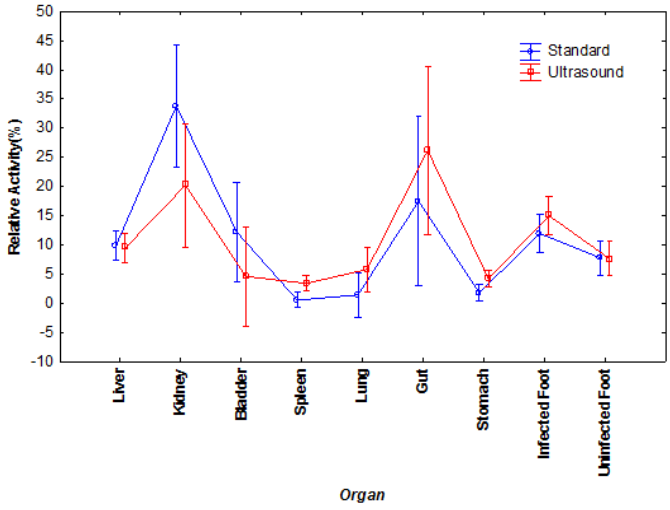


Fig. 4. Mean measured relative activity of the organ of interest, after intravenous injection of 37 MBq (1 mCi) samples which prepared by boiling water bath or ultrasound irradiation methods in various organs (Vertical bars denote 0.95 confidence intervals)

Statistical Analysis

Comparisons of measured relative activity between two methods of labeling were carried out in different rat organs was done using repeated measure design analysis of variance. Statistical significance was considered at p-values less than 5%.

RESULTS

The yield of [^{99m}Tc/Tricine/HYNIC] UBI $_{29-41}$ samples (n= 10) were prepared by boiling water bath as standard method approximately 79.7 ± 1.65 %.

Table 1. The 740MBq $^{99m}TcO_4^-$ (20 mCi) freshly elution solutions of $^{99m}TcO_4^-$ were added to the [Tricine/HYNIC] UBI $_{29-41}$ vials and the mixtures sonicated at 25, 40, 55,70 and 85 ºC in the thermo stated bath (Elma, p == 95 W, made in Germany) for 1,2,3,4 and 5 min. Every experiment was repeated three times and the mean yields of $^{99m}TcO_4^-$, $^{99m}TcO_2$ and ^{99m}Tc-UBI $_{29-41}$ presented

Temperature ºC		Time (Min)				
		1	2	3	4	5
25	$^{99m}TcO_4^-$ %	15.22	18.56	19.27	19.65	20.5
	$^{99m}TcO_2$%	48.36	42.02	37.11	34.95	32.68
	^{99m}Tc-UBI $_{29-41}$ %	33.93	35.95	41.69	43.00	40.27
	$^{99m}TcO_4^-$ %	8.69	7.7	13.53	13.00	14.58
40	$^{99m}TcO_2$%	41.14	39.19	32.24	32.66	34.08
	^{99m}Tc-UBI $_{29-41}$ %	40.27	41.47	45.8	46.34	47.16
	$^{99m}TcO_4^-$ %	9.17	7.7	7.72	8.83	12.18
55	$^{99m}TcO_2$ %	36.18	28.16	32.5	33.11	33.14
	^{99m}Tc-UBI $_{29-41}$%	42.19	54.57	53.87	52.3	51.5
70	$^{99m}TcO_4^-$ %	9.31	9.77	5.27	9.38	8.55
	$^{99m}TcO_2$ %	26.74	15.04	10.48	12.30	19.58
	^{99m}Tc-UBI $_{29-41}$ %	58.00	72.94	82.84	75.34	69.24
85	$^{99m}TcO_4^-$ %	5.57	9.27	9.71	9.8	10.19
	$^{99m}TcO_2$ %	27.04	25.6	25.64	32.89	33.48
	^{99m}Tc-UBI $_{29-41}$ %	61.58	60.44	62.25	53.50	51.64

This finding was very close to the results that we were previously reported (Doroudi and Samaee *et al*., 2015). According to the manufacturer's instructions, this amount of yields are acceptable when are determined by ascending thin layer chromatography. Because the radiotracer complex is large molecule and trailed on silica gel strips, therefore the result of measurement was uncertain to calculate the yields of reactions. ITLC analysis was only considered as primitive criteria in order to find out the suitable conditions for preparation radiotracer samples under ultrasound irradiation technique. Ultrasound irradiation reactions were carried out at 25, 40, 55,70and 85 ºC for 1, 2, 3, 4 and 5 min in order to determine the appropriate condition for reconstitution radiotracer samples.

As it is shown in Table 1, the reaction temperature and time period of ultrasound irradiation had a significant effect on the reaction. When the reaction was carried out at 25, 40, 55, 70 ºC the yield was increased .The yield was increased with increasing temperature and reached to maximum 82.84%. But when the temperature was raised to 85 ºC, the yields dropped to 61.58 %. For this reason the temperature 70 ºC was chosen as the suitable temperature. The time period of reaction was another important factor. The yield was decreased from 82.84 % to 69.24 at 70 ºC when the time of reaction was increased from 3 to 5 min. Therefore, the temperature 70 ºC for 3 min was chosen as suitable condition for preparation of samples due to ultrasound irradiation technique. All radio-HPLC, imaging and quantitative studies have been performed with the infection-seeking samples which were reconstituted by ultrasound irradiation method at 70 ºC for 3 min versus to standard method. As it is stated in Fig2, the retention times of $^{99m}TcO_4^-$ and [^{99m}Tc/Tricine/HYNIC] UBI $_{29-41}$ complex were approximately 4.32 min and 12.67 min respectively in radio-HPLC analysis, when the complexes were prepared by standard method.

The retention times were approximately 4.06 min and 12.64 min when the radiotracer samples were prepared by new developed technique. The retention time of ^{99m}Tc-Ligand samples were identical which were reconstituted by either two above mentioned techniques.

In radio-HPLC investigation of ^{99m}Tc-Ligand demonstrated that the reaction was leaded to a single complex and its retention time was found to be approximately 12 min. This finding demonstrates the successful preparation of [^{99m}Tc/Tricine/HYNIC] UBI $_{29-41}$ complex due to ultrasound irradiation method. The radio labeling yield of ^{99m}Tc-Ligand samples which were prepared by boiling water bath method and measured by radio-HPLC analysis was 89± 1.88 % (n=10). The radio labeling yield of radiotracer samples which were prepared by ultrasound irradiation method was 91.4± 0.35 % (n=10). The radioisotope scintigraphy studies have been performed not only in order to visualize the infected lesion induced in the foot's rat, but also the biodistribution of ^{99m}Tc-Ligand samples in the other organs was assessed in the infected rat. All lesions were induced with S aureus in the left foot of rats in order to exclude any misinterpretation of images. The uptake of radiotracer in the affected food as target or ROI in comparison to the contra lateral healthy side as non target has been considered in all studies.

The visual inspection of images indicated that the specific accumulation of radiotracer complex samples at the septic foci were sufficient to detect the affected region. Images with good quality were obtained in each case and the quality of images did not change over the time. Radiotracer uptake was observed at the infected site in all images. The target to non-target ratio in the radioisotope scintigraphy imaging to localize septic lesions was 2.86 (n= 5, range 2.46.to 3.2 and mean 2=8.6) for samples which were prepared by boiling water bath method. This ratio was 2.88 (n=5, range 2.58 to 3.64 and mean= 2.88) for samples which were prepared by ultrasound irradiation method. Our achievement indicated that the accumulation of ^{99m}Tc-Ligand samples prepared by two above mentioned modalities were identical at the septic lesions induced by S aureus in the rat's foot. Quantitative study has been performed in order to provide further information about the bio distribution of radiotracer samples on the other parts of rat's body. Regardless of method of kit preparation, mean relative activity absorptions among different organs was statistically different ($p<0.0001$). Highest mean relative activity absorptions were seen in kidney and gut. Similar pattern of tissue distribution was seen for both kit preparations.

Difference of mean relative activity of prepared samples by the two techniques was not statistically significant. Although some significant differences were observed between organ distribution of radiotracer samples, mean relative activity of samples in infected and uninfected foots which were prepared by boiling water bath or ultrasound irradiation methods could not be considered significant.

DISCUSSION

^{99m}Tc radioisotope is widely used in diagnostic procedures practically. Its popularity is mainly to the matter that the radioisotope can be readily produced by ^{99}Mo/^{99m}TC generators. It has the ideal γ-ray energy (140keV) which is suitable for gamma camera detection. In addition to the above mentioned factors, the physical half-life is compatible with the biological localization and residence time required for radioisotope scintigraphy imaging. The various complexes of ^{99m}Tc may be formed by interactions between donor electron atoms and empty orbital of reduced ^{99m}Tc.

The structure of ligand must have electron donors such as oxygen, nitrogen and sulfur in order to form bonds between ligand and ^{99m}Tc.^{99m}Tc radioisotope in the $^{99m}TcO_4^-$ form is present in the elution solution which obtained from ^{99}Mo/^{99m}TC generator. The reducing agents such as stannous chloride are commonly used in formulation of radiopharmaceutical kits to reduce ^{99m}Tc from +7 to lower valence state which can be able to react with ligands. The Iranian freeze-dried kit of UBI $_{29-41}$ contains, in vacuum a lyophilized, sterile and pyrogen free mixture of HYNIC-Ubiquicidin$_{29-41}$ 40µg, stannous chloride 40µg and tricine 20mg which facilitates labeling by ligand exchange at elevated temperature. Therefore, only local induced heating due to vibration of ultrasound irradiation instrument without heating was not sufficient to produce ^{99m}Tc-Ligand sample with appropriated yield.

For the presence of electron donating groups, UBI $_{29-41}$ peptide molecule could be coordinated with ^{99m}Tc radioisotope. When the reaction was carried out at70 ºC for 3 min under ultrasound irradiation, the radiotracer samples were produced with suitable amount of yields. The nature of this kind of bonding is not strong as covalent bonding. For this reason, elevated temperature above 70 ºC could influence on the nature of this interaction or degrade the structure of infection-seeking radiotracer complex samples under ultrasound irradiation. Therefore, the yield of reaction could be considerable decreased. The radio-HPLC and ITLC studies have shown the successful reconstitution radiotracer samples by new developed modality. According to the literature, the microwave oven heating as an alternative technique has been previously recommended for the preparation of MIBI kits (Gagnon *et al*., 1991). The microwave oven technique has been preformed not only for synthesis of MIBI (Lima *et al*., 2005) but also for reconstitution of ^{99m}Tc-MIBI complex samples (Hung *et al*., 1991). The time for labeling of Sestamibi could be reduced to 10 seconds by the microwave heating technique. In spite of rapid reconstitution of Sestamibi by microwave oven heating, this method has following precaution factors that must be considered. Geometry of samples inside the device is important.

There is a potential risk of sparking for the presence of metal cap of the vial. Any residual gas could be left in the head space of the vial caused an ejection of the rubber stopper due to the excess steam pressure built up the vial. Microwave oven instruments with digital control panel are more suitable for setting short heating time (i.e., 10s) since they must be accurately set at the required heating period. The loss or variation of microwave oven output and frequency related to extensive use of the device should be evaluated on a long-term usage. Any techniqal error in setting the instrument heating time below or beyond the predetermined time may result in the radiotracer solution being rendered unsuitable for clinical application. Therefore, the microwave oven technique is not routinely used in clinical practice for the above mentioned precaution factors. On the basis of results have been obtained from our approach, the preparation of the infection-seeking radiotracer samples were carried out in milder condition by ultrasound irradiation versus boiling water bath technique.

In addition to the above mentioned factor, the outcome of our study indicated that the preparation of ^{99m}Tc-Ligand radiotracer samples by ultrasound irradiation technique is reliable and reproducible method to facilitate the reconstitution [^{99m}Tc/Tricine/HYNIC] UBI $_{29-41}$ kits. The preparation of radiotracer samples by new developed technique has the following advantages. The geometry of samples in instrument was not important factor. The time labeling process of UBI $_{29-41}$ by freshly eluted solution of pertechnetate -99m sodium by ultrasound irradiation method versus boiling water bath method was considerably reduced. The infection-seeking radiotracer samples were prepared in sufficient amounts and good yields under the milder condition. There was not potential risk of sparking for the presence of the metal cap of the vial inside the ultrasound apparatus.

The potential risk of absorbed ionization irradiation to the personnel who are working in nuclear medicine department could be declined significantly. In addition to the above mentioned advantages, energy consumption can be saved by ultrasound irradiation technique in comparison to the conventional method. The new developed technique for preparation [^{99m}Tc/Tricine/HYNIC] UBI $_{29-41}$ can be carried out in any nuclear medicine department and permitted a fast and reliable method to make radiotracer samples. The ultrasound irradiation instruments with different powers are present in the markets. The ultrasound irradiation technique should be set out in nuclear medicine departments in order to find out the suitable conditions from the aspect of temperature and time period of heating according to the power of instrument. To facilitate the preparation of ^{99m}Tc-Ligand samples, it is necessary that the legal considerations of using this new developed technique must be judged and approved.

Conclusion

[^{99m}Tc/Tricine/HYNIC]UBI $_{29-41}$ complex samples can be prepared efficiently with appropriate yields by ultrasound irradiation technique. Green chemistry can open a new field in nuclear medicine practically. Ultrasound irradiation technique can be recommended for the reconstitution of the

radiopharmaceutical complex samples where the preparations of kits are time-consuming.

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Abbreviations

Bq: Becquerel , Mo: Molybdenium , TcO_4: Pertechnetate , Tc: Technetium, UBI $_{29-41}$: Ubiquicidin

Competing interests

The authors declare that they have no competing interests

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