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PREPARATION OF ^{99m}Tc-TRICINE-EDDA-HYNIC-FOLATE, A POTENTIAL RADIOPHARMACEUTICAL FOR RADIODIAGNOSIS OF FOLATE RECEPTORS OVER EXPRESSED CANCERS

Martalena Ramli, Rien Ritawidya, Cecep Taufik Rustendi, Titis Sekar Humani and Widyastuti Widjaksana
Radioisotope and Radiopharmaceutical Technological Centre (*PRR*)*-BATAN*
Kawasan Puspiptek, Serpong 15314, Tangerang Selatan
E-mail: marta_r@batan.go.id

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ABSTRACT

PREPARATION OF ^{99m}Tc-TRICINE-EDDA-HYNIC-FOLATE, A POTENTIAL RADIOPHARMACEUTICAL FOR RADIODIAGNOSIS OF FOLATE RECEPTORS OVER EXPRESSED CANCER. Folate receptors (FRs) have been reported to be over expressed on various types of cancers. Therefore, it would be possible for its ligand in this case folic acid, also known as vitamin B_9, to be used as delivery agent for diagnosis and therapy of FRs over expressed cancers. The aim of this project was to prepare ^{99m}Tc radiolabeled folic acid via 6-hydrazinonicotinamido-hydrazido (HYNIC) in the form of ^{99m}Tc-tricine-ethylenediamine diacetate-HYNIC-folate (^{99m}Tc-tricine-EDDA-HYNIC-folate), which was expected to be potential for radiodiagnosis of the FRs over expressed cancers. Preparation of ^{99m}Tc-tricine-EDDA-HYNIC-folate was initiated by preparation of HYNIC-folate by reacting of folate-γ-hydrazide with 6-chloronicotinic acid NHS ester which was then followed by addition of hydrazine-hydrate. The HYNIC-folate was recovered in its HCl salt-form which was then formulated to form a freeze dried kit which consisted of HYNIC-folate, tricine and EDDA (co-ligands) and Sn(II) as reducing agent. The formation of ^{99m}Tc-tricine-EDDA-HYNIC-folate was carried out by addition of ^{99m}Tc into tricine-EDDA-HYNIC-folate freeze dried kit which resulted in ^{99m}Tc-tricine-EDDA-HYNIC-folate with radiochemical purity of 97.0 ± 1.8% met with the requirement of a good radiopharmaceutical (≥ 90%). The stability test showed that the ^{99m}Tc-tricine-EDDA-HYNIC-folate was still intact (radiochemical purity ~ 95%) when stored at 37 °C for four hours.

Keywords: Folate receptors, Cancer, Diagnostic radiopharmaceutical, ^{99m}Tc-tricine-EDDA-HYNIC-folate

ABSTRAK

PREPARASI *^{99m}Tc-TRICINE-EDDA-HYNIC-FOLATE*, RADIOFARMAKA POTENSIAL UNTUK RADIODIAGNOSIS KANKER *OVER EXPRESSED* RESEPTOR FOLAT. Reseptor Folat (*FRs*) dilaporkan *over expressed* pada beberapa jenis kanker. Asam folat atau yang juga dikenal sebagai vitamin B_9 merupakan ligan dari *FRs*, sehingga asam folat memungkinkan untuk digunakan sebagai pembawa radionuklida untuk radiodiagnosis atau radioterapi kanker *over expressed FRs*. Tujuan penelitian ini adalah untuk menyiapkan asam folat bertanda ^{99m}Tc dengan menggunakan *6-hydrazinonicotinamido-hydrazido (HYNIC)* sebagai *bifunctional chelating agent* dalam bentuk *^{99m}Tc-tricine-ethylenediamine diacetate-HYNIC-folate (^{99m}Tc-tricine-EDDA-HYNIC-folate)* yang diharapkan potensial untuk radiodiagnosis kanker *over expressed FRs*. Preparasi *^{99m}Tc-tricine-EDDA-HYNIC-folate* diawali dengan penyiapan *HYNIC-folate* dengan mereaksikan *folate-γ-hydrazide* dengan *6-chloronicotinic acid NHS ester* yang diikuti dengan penambahan *hydrazine-hydrate*. *HYNIC-folate* yang terbentuk kemudian diisolasi dalam bentuk garam *HYNIC-folate-HCl* yang kemudian diformulasi dalam bentuk *freeze dried kit* yang mengandung *HYNIC-folate*, *tricine* dan *EDDA (co-ligands)* serta Sn(II) sebagai reduktor. Penyiapan *^{99m}Tc-tricine-EDDA-HYNIC-folate* dilakukan dengan menambahkan ^{99m}Tc pada *freeze dried kit tricine-EDDA-HYNIC-folate* yang menghasilkan *^{99m}Tc-tricine-EDDA-HYNIC-folate* dengan kemurnian radiokimia 97,0

± 1,8% yang memenuhi persyaratan kemurnian radiokimia suatu radiofarmaka yang baik (≥ 90%). Hasil uji stabilitas *^{99m}Tc-tricine-EDDA-HYNIC-folate* memperlihatkan bahwa radiofarmaka ini masih tetap utuh (kemurnian radiokimia ~ 95%) setelah disimpan pada 37 °C selama 4 jam.

Kata kunci: Reseptor folat, Kanker, Radiofarmaka diagnosis, *^{99m}Tc-tricine-EDDA-HYNIC-folate*

INTRODUCTION

Cancer has now become one of health problems around the world. Based on Globocan report in 2012 there were 14.090 millions of new cancer cases with number of deaths of 8.201 millions out of 7.054446 billions total world population. Five most frequent for the above-mentioned cancers were lung, breast, colorectal, prostate and stomach cancers. In case of Indonesia, in 2012 Globocan reported there were around 299.7 thousands of new cancer with a number of deaths of around 194.5 thousands out of around 245 millions of population. Five most frequent for the above-mentioned cancers were breast, lung, colorectal, cervix uteri stomach and liver cancers [1]. These statistics show that cancer has become a heavy burden to our society welfare physically and emotionally.

Radiopharmaceutical is one of many modalities that have been used in cancer management. It can be used as radiodiagnostic or radioimaging and/or radiotherapeutic agents. In recent years there has been a tendency to use targeted radiopharmaceuticals in order to avoid unnecessary radiation to normal cells. This can be performed by exploiting a phenomenon of an affinity between target on surface of cancer cells and its associated ligand. There are several targets that can be exploited for the above mentioned purposes. Some of them are CD20, CD30, CD52, epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), human epidermal receptor type 2 (HER-2) and folate receptors (FRs) [2,3]. Suitable ligands for the above-mentioned targets could be biomolecules such as monoclonal antibodies, peptides, hormones, vitamins and other biomolecules. For a targeted radiopharmaceuticals these ligands are used to deliver the conjugated or attached probe in form of radionuclides which emits a γ gamma radiation and/or α or β particles.

Folic acid, which is also known as vitamin B_9 and has a molecular weight of 441 Da, is an essential vitamin required for proliferation and maintenance of mammalian cells [4-6]. Folic acid is internalised by normal mammalian cells via either a low affinity reduced folate carrier (RFC) or proton-coupled folate transporter (PCFT). Unlike folate, conjugated folate does not show any affinity toward RFC or PCFT. However, conjugated folates do have high affinity (K_d: 10^{-10} M) toward folate receptors (FRs) which are over expressed and accessible on several cancers such as ovary, lung, breast, kidney, brain, endometrial, colon and hematopoietic cells of myelogenous origin, also on activated macrophages and proximal tubule cells of the kidney. Therefore, folate based radiopharmaceuticals are expected to be versatile and target specific for diagnosis and/ or therapy of FRs over expressed cancers.

There have been several investigations related to the use of folate based radiopharmaceutical for radiodiagnostic agents. These include ^{111}In diethylene-triamine pentaacetic acid (DTPA)-folate and ^{99m}Tc 6-hydrazinonicotinamido-hydrazido (^{99m}Tc-HYNIC)-folate with co-ligands of tricine and trisodium triphenylphosphine-3,3,3''-trisulfonate (TPPTS) [7-9]. Pre-clinical and clinical tests showed that these agents were potential for imaging of FRs over expressed positive cancers.

However, there have been a few drawbacks in the use of the above-mention agents. These included the use of ^{111}In which was considered less suitable for radiodiagnostic agent due to its Auger and Coster Kronig electrons and preparation of radiolabeling DTPA-folate with ^{111}In which involved two-step reaction where ^{111}In first has to be complexed with citrate. The resulted ^{111}In-citrate was then reacted with DTPA-folate to form ^{111}In- DTPA-folate [7,10]. In case of ^{99m}Tc-tricine-TSPP-HYNIC-folate, radiolabeling of tricine-TSPP-HYNIC-folate with ^{99m}Tc can be performed in one step reaction which is much preferred by the users [9]. However, production of ^{99m}Tc-tricine-TPPTS-HYNIC-folate which has a great potential for radiodiagnostic agent of FRs over expressed cancers has been threatened due the the difficulty in procurement of its co-ligands TPPTS.

The purpose of this study was therefore to prepare ^{99m}Tc-HYNIC with co-ligand such as tricine-ethylendiaminediaceticacid (EDDA) which can procured easily and expected to provide a stable ^{99m}Tc-HYNIC-folate with acceptable radiochemical purity (> 90%).

EXPERIMENTAL METHOD

Material and Equipment

Chemicals and materials used in this project included folate-γ-hydrazide provided by Chiroblock (Germany), dicyclocarbodiimide (DCC), N-hydrosuccinimide (NHS), dimethylsulfoxide (DMSO) and hydrazine hydrate, tricine, and ethylendiaminediacetic acid (EDDA) were obtained from Aldrich Chemical. Tc-99m was generated from ^{99}Mo/^{99m}Tc Generator (PT. BATAN Teknologi, Indonesia). Silica gel impregnated glass fibre sheets/

instance thin layer chromatography-silica gel (ITLC-SG) were obtained from Pall.

Equipment used in this research included magnetic stirrer (Cimarec), centrifuge (Hettich-EBA 8S), FT-IR Spectrometer (Jasco-410), thin layer chromatographic scanner (Bio Scan), dose calibrator (Capintec - CRC-15R) and thermomixer (Bio Rad).

Synthesis of 6-Hydrazinonicotinamido-Hydrazido (HYNIC)-Folate (5)

The synthesis scheme of HYNIC-folate is shown in Figure 1. This synthesis procedure is performed in similar manner to that of Guo *et al* [9]. HYNIC-folate was prepared by reacting folate-γ-hydrazide (**1**) with 6-chloronicotinic acid NHS-ester (**3**). 6-Chloronicotinic acid NHS-ester (**3**) was prepared by dissolving 6-chloronicotinic acid (**1**) (200 mg) in 2 mL DMSO which was followed by addition of NHS (**2**) (146 mg) and DCC (261 mg) (Figure 2). The reaction mixture was left to react for four hours at ambient temperature with stirring and protected from light. Dicyclohexylurea, by product, was removed by a pellet formation using a centrifuge. After pellet removal, one mL of reaction mixture was withdrawn and then added to folate-γ-hydrazide (50 mg) in 2 mL of DMSO. Reaction mixture was left to react for four hours at ambient temperature with stirring to form folate-6-chloronicotinamide (**4**) which was then followed by addition of hydrazine-hydrate (200 mg). The reaction mixture was left to react overnight which was followed by addition of 100 µL of 0.5 N HCl to produce HYNIC-folate.HCl. The salt was then separated by centrifugation which was then recovered and washed with ethanol (4 times). Finally, the salt was washed with ether and left to dry under vacuum at - 20° C. The product was then analysed by a FT-IR Spectrometer.

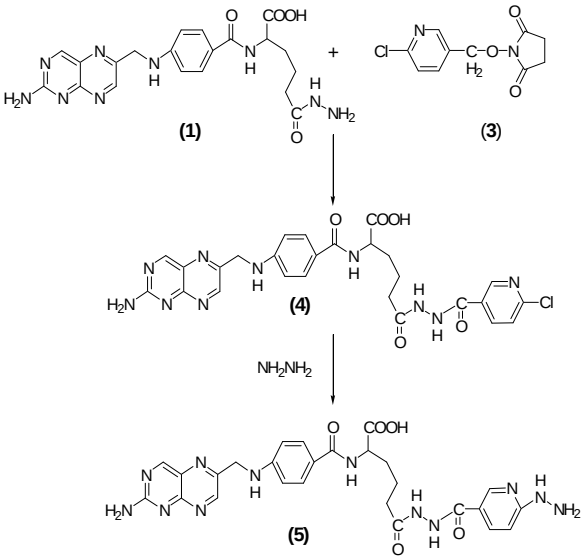

Figure 1. Preparation scheme of HYNIC-folate [9]

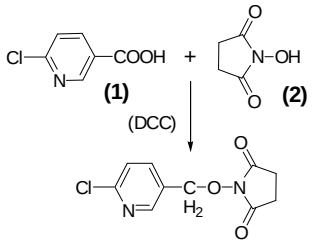

Figure 2. Preparation scheme of 6-chloronicotinic acid NHS ester [9].

Formulation of Tricine-EDDA-HYNIC-Folate Cold Kit

Tricine-EDDA-HYNIC-folate freeze dried kit was prepared by adding 800 µL of HYNIC-folate solution (0.5 mg/ mL) in 0.1 M phosphate buffer pH 6 into a mixture of tricine (100 mg) and EDDA (200 mg) in 500 µL H_2O. Aliquot (500 µL) of $SnCl_2.2H_2O$ (1 mg/mL) in N_2-saturated H_2O was then added which was followed by pH adjustment to 7 with addition of 1N NaOH. The solution diluted with H_2O to give a final volume of 10 mL. The solution was then flushed with N_2 for five minutes which was then followed by dispensing to a series of 10 mL vials (1 mL/vial) and freeze drying for 24 hours.

Radiolabeling of Tricine-EDDA-HYNIC-Folate with ^{99m}Tc

Radiolabeling tricine-EDDA-HYNIC-folate with ^{99m}Tc was performed by firstly reconsituting the tricine-EDDA-HYNIC-folate freeze dried kit with one mL of 0.1 M phosphate buffer pH 7. Tc-99m (5 – 10 mCi) which was generated from ^{99}Mo/^{99m}Tc-Generator was added and the reaction mixture was heated at 80 °C for 30 minutes. Radiochemical purity of ^{99m}Tc-tricine-EDDA HYNIC-folate was then determined by two thin layer chromatographic (TLC) systems using ITLS-SG as a stationary phase with acetone and saline solution (0.9% NaCl) as mobile phases.

Determination of Radiochemical Purity of ^{99m}Tc-Tricine-EDDA-HYNIC-Folate

Radiochemical purity of ^{99m}Tc-tricine-EDDA HYNIC-folate was determined by two TLC systems: a) ITLS-SG as a stationary phase and acetone as mobile phase; and b) ITLS-SG as a stationary phase and saline solution (0.9% NaCl) as mobile phase. ITLC-SG sheets were cut into strips (0.8 x 10 cm). The strips were activated by heating at 110 °C for at least 10 minutes before their use. Aliquot of the reaction mixture was spotted 1 cm from the bottom of four ITLC-SG strips. Two ITLC-SG strips were developed in acetone and the other two were in saline solution (0.9% NaCl). When the solvent front was within 0.5 cm of the top of the strips, they were then

removed and allowed to dry on blotting paper. The strips were then scanned by TLC scanner. The percentage of complex ^{99m}Tc-tricine-EDDA-HYNIC-folate was calculated by deducting the percentage of radioactivity which was associated with ^{99m}Tc-tricine-EDDA-HYNIC-folate with the percentage of radioactivity which was associated with free ^{99m}Tc and $^{99m}TcO_2$.

Determination of Stability of ^{99m}Tc-Tricine-EDDA HYNIC-Folate

^{99m}Tc- tricine-EDDA-HYNIC-folate was stored at 37 ^{o}C for up to four hours. Aliquots were removed at one, two, three and four hours and then analysed for their radiochemical purity by two TLC systems : a). ITLC-SG as a stationary phase and acetone as mobile phase; and b). ITLC-SG as a stationary phase and saline solution (0.9% NaCl) as mobile phase.

RESULTS AND DISCUSSIONS

HYNIC-folate (5) was prepared by reacting folate γ-hydrazide (1) with 6-chloronicotinic acid NHS ester (3) (Figure 1) where 6-chloronicotinic acid NHS ester (3) was prepared by reacting 6-chloronicotinic acid (1) with NHS (2) in the presence of DCC (Figure 2).

Figure 3(a) and 3(b) show the FT-IR spectrum of the formed HYNIC-folate and folate γ-hydrazide (precursor) respectively. The formation of HYNIC-folate could be recognised by absorbance on the 900-675 cm^{-1} region (Figure 3(a)). This region is the most characteristic absorbance for polynuclear aromatics which are generated from C-H out of plane bending [11]. It can be seen in Figure 3(a), there are three strong bands on the region between 900 - 675 cm^{-1} that correlate with the number of adjacent hydrogen atom on the rings. For comparison, on Figure 3(b) (FT-IR spectrum of folate γ-hydrazide) there are relative weak bands on the 900 - 675 cm^{-1} region. These indicated that there were less hydrogen atom on the rings on the latter (folate γ-hydrazide) compared to that of the former (HYNIC-folate).

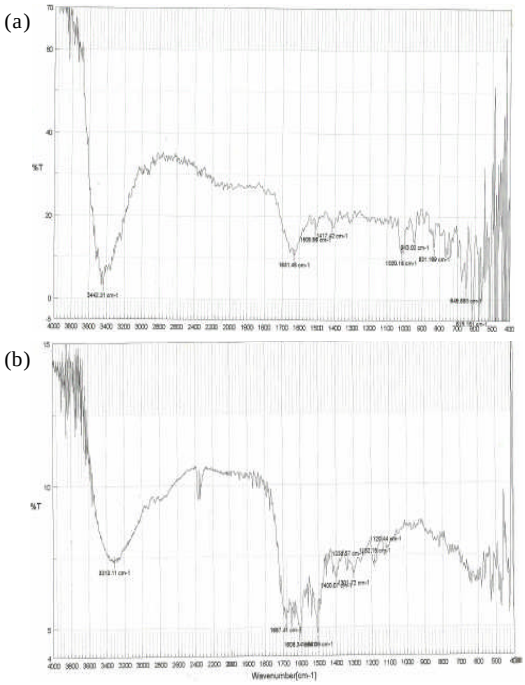

Figure 3. FT-IR spectrum of HYNIC folate (a) and folate g-hydrazide (b)

HYNIC was firstly introduced by Abrams, *et al*. [12] as bifunctional chelating agent (BFCA) or ligand for tagging a biomolecule (BM) with ^{99m}Tc and acts as a monodentate ligand. Therefore, when it is used for chelating ^{99m}Tc (which is able to receive seven pairs of lone electrons), it has to be accompanied by co-ligand in order to stabilize the resulting ^{99m}Tc-HYNIC complex. There were several reports related to the use of HYNIC as BFCA for radiolabeling of BM with ^{99m}Tc where tricine, TSPP, or EDDA uses as co-ligand/s [9,13,14]. Preparation of ^{99m}Tc-HYNIC-BM where tricine used as co-ligand has been reported to be relatively simple. It could be performed at room temperature (20 °C) with reaction time of 10 minutes which resulted in ^{99m}Tc-tricine-HYNIC-BM with radiochemical purity > 99% [14]. The resulted ^{99m}Tc-tricine-HYNIC-BM complex however was reported to be unstable and also presence in multiple species in solution due to different bonding modalities of HYNIC and co-ligands. On the other hand, radiolabeling of HYNIC-BM such as HYNIC-folate and HYNIC-Duramycin with ^{99m}Tc where tricine along with TPPTS used as co-ligand resulted in stable ^{99m}Tc-tricine-TPPTS-HYNIC-folate and ^{99m}Tc-tricine-TPPTS-HYNIC-Duramycin complexes respectively [9,13]. The radiolabeling procedure for the above-mentioned HYNIC-BM was relatively simple and gave radiochemical purity > 90%. However, the difficulty in procurement of TPPTS has leaded to the use of other co-ligand for radiolabeling HYNIC-BM with ^{99m}Tc.

EDDA is one of co-ligands that has ben explored for radiolabeling of HYNIC-BM [15,16]. Radiolabeling HYNIC-folate with ^{99m}Tc with co-ligand EDDA which was carried out in our laboratory however, only resulted in ^{99m}Tc-EDDA-HYNIC-folate with an average radiochemical purity of 75% which is not met with the radiochemical purity of a good radiopharmaceutical (> 90%) [17]. This result was in agreement with report on radiolabeling of HYNIC-BM with ^{99m}Tc where EDDA used as a sole co-ligand [15]. This procedure is not preferred by users as ^{99m}Tc-EDDA-HYNIC-folate had to be purified to increase its radiochemical purity (> 90%) prior its use. Nevertheless, purified ^{99m}Tc-EDDA-HYNIC-folate reported to be more stable compared to ^{99m}Tc-tricine-HYNIC-folate. Referring to the simple procedure for radiolabeling HYNIC-BM with ^{99m}Tc where tricine and TSPP used as co-ligand, it was then a new approach for radiolabeling of HYNIC-BM with ^{99m}Tc being developed.

In this work, radiolabeling of HYNIC-folate with ^{99m}Tc was peformed by using tricine along with EDDA

as co-ligands. Freeze dried kits, which contained HYNIC-folate, tricine, EDDA and certain amount of Sn(II) that required for reducing of Tc(VII) to Tc(V), were prepared. Radiolabeling of the above-mentioned kit was carried out by firstly reconstituting the freeze dried kit with 0.1 M phosphate buffer pH 7 which was then followed by adition of ^{99m}Tc (5 – 10 mCi). Reaction mixture was left to react at 80 ^{o}C for 30 minutes before aliquots were taken for radiochemical purity test by using two TLC systems.

Based on the ITLC-SG–acetone system (Figure 5(a)) the ^{99m}Tc-tricine-EDDA-HYNIC-folate and $^{99m}TcO_2$ would have an Rf < 0.5 and the free $^{99m}TcO_4^-$ would have an Rf >0.5. The $^{99m}TcO_2$ colloid that might be produced in radiolabeling process due to an excess of Sn(II) was measured by using ITLC-SG-saline system. Based on this TLC, the colloidal $^{99m}TcO_2$ would have an Rf < 0.5 while the ^{99m}Tc- tricine-EDDA-HYNIC-folate and free $^{99m}TcO_4^-$ would have an Rf > 0.5 (Figure 5(b)). It can be seen on Figure 5(a) that there was 97.26% of the ^{99m}Tc- tricine-EDDA-HYNIC-folate and 2.74% $^{99m}TcO_2$ after radiolabeling process. From Figure 5(b) it can be seen that the percentage of $^{99m}TcO_2$ in the ^{99m}Tc-tricine-EDDA-HYNIC-folate product was of 2.06%. The tabulated of RFs of each component resulted from radiolabeling of the ^{99m}Tc-tricine-EDDA-HYNIC-folate with ^{99m}Tc determined with two TLC systems is shown in Table 1.

The percentage of ^{99m}Tc-tricine-EDDA-HYNIC-folate formed, expressed as radiochemical purity, calculated by deducting the percentage of radioactivity

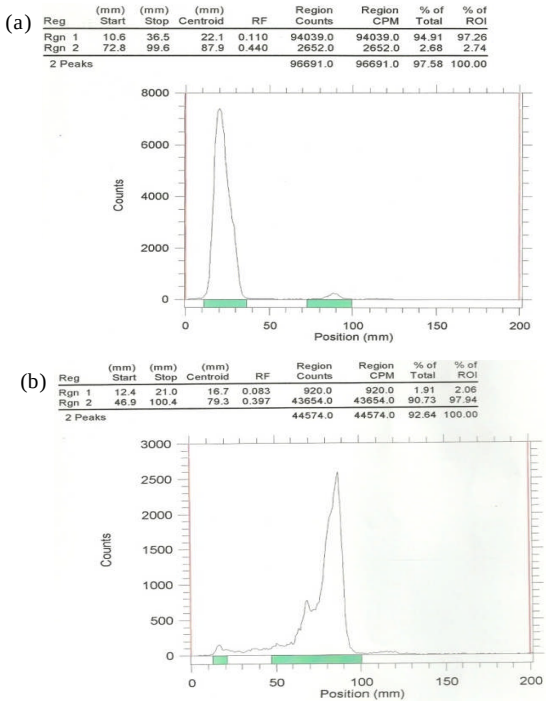

Figure 5. Radiochromatograms of ^{99m}Tc-HYNIC-folate:

Note: (a) Developed with acetone and (b) Developed with saline solution.

Table 1. The RFs of each component resulted in radiolabeling of ^{99m}Tc-tricine-EDDA HYNIC-folate with ^{99m}Tc (Determined with two TLC sytems)

Fig	TLC System	Rf > 0.5	Rf < 0.5
5(a)	ITLC-SG/ Acetone	Free ^{99m}Tc (2.74%)	^{99m}Tc- tricine-EDDA-HYNIC-folate + $^{99m}TcO_2$ (97.26%)
5(b)	ITLC-SG/ 0,9% NaCl	^{99m}Tc- tricine-EDDA HYNIC-folate + free ^{99m}Tc (97.94%)	$^{99m}TcO_2$ (2.06%).

which was associated with ^{99m}Tc-tricine-EDDA-HYNIC-folate with the percentage of radioactivity which was associated with free $^{99m}TcO_4^-$ and $^{99m}TcO_2$. The radiochemical purity of ^{99m}Tc-tricine-EDDA-HYNIC-folate based on the above chromatograms (Figure 5(a) and 5(b)) and Table 1 calculated by deducting percentage of [% ^{99m}Tc- tricine-EDDA-HYNIC-folate + % $^{99m}TcO_2$] from radiochromatogram (a) with percentage of [$^{99m}TcO_2$] from radiochromatogram (b). The radiochemical purity of ^{99m}Tc-tricine-EDDA HYNIC-folate based on the above-mention calculation was found to be 95.2%. This value exceeded the radiochemical purity required for good radiopharmaceuticals (> 90%). Several repetitions of radiolabeling of HYNIC-folate kit with ^{99m}Tc were successfully produced ^{99m}Tc-tricine-EDDA-HYNIC-folate with an average radiochemical purity of 97.0 ± 1.8%. Based on these results, it appeared that the formulation of Tc-HYNIC-folate kit containing tricine and EDDA as co-ligands was suitable for preparation of ^{99m}Tc-HYNIC-folate.

There have been a few reports regarding the nature of formed complexes when two co-ligands used at the same time in radiolabeling of HYNIC-BM with ^{99m}Tc [13,14]. In case radiolabeling of HYNIC-BM with ^{99m}Tc where tricine and TSPP used as co-ligands, it was reported that these co-ligands were fully participated in forming the ^{99m}Tc-tricine-TPPTS-HYNIC-BM complex [13]. On the other hand, radiolabeling of HYNIC-BM with ^{99m}Tc where tricine and EDDA as co-ligands was reported to solely form of ^{99m}Tc-EDDA-HYNIC-BM complex. In this radiolabeling it was suggested that at the early stage of radiolabeling ^{99m}Tc-tricine-HYNIC-BM was formed which was followed by trans-chelation of tricine with EDDA at a later stage of reaction [14].

The stability of ^{99m}Tc-tricine-EDDA HYNIC-folate, measured as radiochemical purity, was observed from radiochemical purity of ^{99m}Tc tricine-EDDA HYNIC-folate which was stored at 37 °C for up to four hours. The radiochemical purity of ^{99m}Tc-tricine-EDDA- HYNIC-folate was measured by TLC using ITLC-SG-acetone and

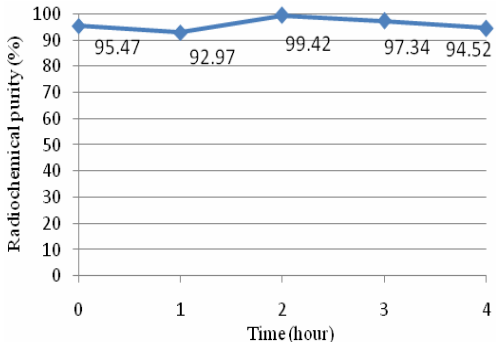

Figure 6. The stability of ^{99m}Tc-tricine-EDDA HYNIC-folate

Note: Stored at 37 °C(indicated as radiochemical purity)

ITLC-SG-saline systems. Figure 6 shows the radiochemical purity of ^{99m}Tc-tricine-EDDA-HYNIC-folate over four hours of observation. It can be seen on Figure 6 that the radiochemical purity of ^{99m}Tc-tricine-EDDA-HYNIC-folate was still high (>90%), which indicated that ^{99m}Tc-tricine-EDDA-HYNIC-folate was still intact after four-hours.

CONCLUSIONS

Formulation of freeze dried kit for preparation of ^{99m}Tc-tricine-EDDA-HYNIC-folate, which consisted of HYNIC-folate, tricine, EDDA and Sn(II) was prepared successfully. Preparation of kits were initiated by preparation HYNIC-folate which was prepared by reacting folate γ-hydrazine with 6-chloronicotinic acid NHS ester. The prepared HYNIC-folate was then formulated in order to obtain HYNIC-folate kit which consisted of HYNIC-folate, tricine and EDDA co-ligands, and Sn(II). The radiolabeling of these kits with ^{99m}Tc every time readily produced ^{99m}Tc-tricine-EDDA-HYNIC-folate with radiochemical purity of 97.0 ± 1.8% which met with the requirement of a good radiopharmaceutical. The stability test showed that the ^{99m}Tc-tricine-EDDA-HYNIC-folate was still intact (radiochemical purity ~ 95%) when stored at 37 °C for up to four hours.

In order to use ^{99m}Tc-tricine-EDDA-HYNIC-folate as radiodiagnostic agent for FR over expressed cancers, further studies which include serum stability, lipophilicity, biodistribution and clearance on normal mice and/or xenograph, and on normal and cancer patients need to be performed.

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