2009 International Nuclear Atlantic Conference - INAC 2009
Rio de Janeiro, RJ, Brazil, September27 to October 2, 2009
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: **978-85-99141-03-8**

RADIOLABELING, QUALITY CONTROL AND RADIOCHEMICAL PURITY ASSESSMENT OF ^{99m}Tc-HYNIC-TOC

Laura T. U. H. Melero[1], Elaine. B. Araújo[1] and Jair Mengatti[1]

[1] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
laura_ueda@yahoo.com.br

ABSTRACT

Somatostatine receptors are widely expressed by several tumors, especially of the neuroendocrine origin. *In vivo* images of these tumors using radiolabeled somatostatine analogues became a useful clinical tool in oncology. The aim of this work was the radiolabeling of the somatostatine analogue HYNIC-TOC with ^{99m}Tc as well as the evaluation of the radiochemical stability and quality control of labeled complex. ^{99m}Tc-HYNIC-TOC was produced by labeling conditions using 20 μg of peptide, 20 mg of tricine and 10 mg of EDDA as coligands, 1110 MBq of ^{99m}Tc (^{99}Mo-^{99m}Tc IPEN-TEC generator) and 15 μg of $SnCl_2.2H_2O$. The reaction proceeds for 10 minutes at boiling water bath. Radiochemical purity of labeled preparation was evaluated by different chromatographic systems: ITLC-SG in methanol:ammonium acetate (1:1); TLC-SG in sodium citrate buffer 0.1 N pH 5.0 and methylethylketone, and HPLC employing column C-18, 5 μm, 4.6 mm x 250 mm, UV (220 nm), radioactivity detectors, 1 mL/minute flow of acetonitrile and trifluoroacetic acid solution 0.1 %. Labeled compound has been found radiochemically stable for 5 hours and radiochemical purity was higher than 90 %. The thin layer chromatographic systems enabled the separation of radiochemical species presented in the labeled mixture as well as HPLC system. The labeling procedure studied resulted in high radiochemical yield and easy preparation. Future works include the preparation of a lyophilized reagent to make feasible the preparation of ^{99m}Tc-HYNIC-TOC at nuclear medicine services in order to study the clinical potential of the radiopharmaceutical in diagnostic and staging of neuroendocrine tumors.

1. INTRODUCTION

The somatostatine is a peptide with fourteen or twenty eight aminoacids, multifunctional, synthesized by hipotalamo and pancreas, acting as neurotransmitter in central nervous system and as hormone in other locals. Five types of somatostatine receptor (SSTR) were identified (sstr1-sstr5). The superexpression of somatostatine receptors in tumor cells is a hundred times over than in normal cells, considering neuroendocrine tumors as carcinoides, pancreatic island cell tumor, small cell lung cancer and some thyroid carcinomas. The somatostatine shows affinity for all receptors while synthetic analogs show a substantial variation. The octreotide (TOC) was the first somatostatine synthetic analog introduced in clinical use [1, 2, 3].

It did many attempts to development of somatostatine analogs labeled with ^{99m}Tc (technetium-99m) using a variety of chelant systems until development of the HYNIC-D-Phe^1-Tyr^3-octreotide (6-hydrazino nicotinic acid-D-Phe^1-Tyr^3-octreotide) (Fig. 1), employing tricine and EDDA (ethylenediamine-N,N´-diacetic acid) as coligands, showing a maintenance

of *in vivo* affinity in rats brain cortex membranes and promising patterns of biodistribution *in vivo* in animals with induced tumors, and high like tumor-kidney and tumor-live relation [4].

The imaging of neuroendocrine tumors has become one of the most significant areas in nuclear oncology. Imaging with ^{99m}Tc-HYNIC-TOC showed a greater tumor-to-nontumor ratio and greater uptake. Decristoforo et al. compared the clinical efficacy of ^{99m}Tc-HYNIC-TOC for adequate imaging with commercially available ^{111}In-DTPA-octreotide in patients with various neuroendocrine tumors and found that the ^{99m}Tc labeled TOC had higher tumor-to-background ratios and provided superior scans [5, 6].

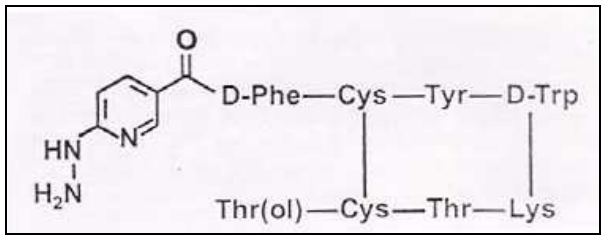

Figure 1. HYNIC-octreotide structure[8]

About 80 % of the radiopharmaceuticals used in nuclear medicine are ^{99m}Tc labeled compounds commercially available and prepared with ^{99m}Tc from ^{99}Mo-^{99m}Tc (molibdenium-99-technetium-99m) generators with low cost. The ^{99m}Tc shows favorable characteristics for application in diagnostic procedures: 6 hours of short half-life, gamma rays emission of 140 keV ideal for scintigraphy images proceedings in gamma chamber or in SPECT (Single Photon Emission Computed Tomography) in nuclear medicine and no particulate emission [7, 8].

The aim of this study was the radiolabeling of the somatostatine analogue HYNIC-TOC with ^{99m}Tc as well as the evaluation of the radiochemical stability of labeled complex.

2. MATERIAL AND METHODS

2.1. Reagents and solvents

All reagents employed were purchased from Sigma-Aldrich in high pure grade. Sodium pertecnetate solution was obtained from IPEN-TEC ^{99}Mo-^{99m}Tc generator (IPEN-CNEN/SP) and HYNIC-octreotide was obtained from Pichem.

2.2. Labeling protocol

In the reaction vial 20 μg of HYNIC-TOC in ethanol 10 % was introduced followed by 10 mg of EDDA in NaOH 0.1 N, 20 mg of tricine in sodium phosphate buffer 0.2 N pH 6.2, 1110 MBq (30 mCi) of $^{99m}TcO_4^-$, 15 μg of $SnCl_2.2H_2O$ in HCl 0.1 N and the reaction mixture was heated in boiling water bath for 10 minutes [8, 9, 10, 11].

2.3. Quality control of ^{99m}Tc-HYNIC-TOC

Radiochemical purity analysis was assessed with two different chromatographic approaches: High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC), using silica gel plates (TLC-SG and ITLC-SG). HPLC technique was used also to validate TLC approach.

The radiochemical purity of ^{99m}Tc-HYNIC-TOC were determined by HPLC with reversed phase C18 column (4.6 mm x 250 mm) using ACN/0.1 % TFA gradient as a mobile phase at a 1 mL/min flow rate. ^{99m}Tc-HYNIC-TOC was monitored by radioactivity detector and UV detector (220 nm).

The radiochemical purity of ^{99m}Tc-HYNIC-TOC determined by ITLC-SG and TLC-SG strips, employed methanol:ammonium acetate (1:1), methylethylketone and sodium citrate buffer 0.1 N pH 5.0 as a mobile phase. Strips were analyzed by gamma counter (Table 1).

Table 1. Chromatographic system and relation front (Rf) of radiochemical species

Radiochemical species	Stationary phase	Mobile phase	Rf
$^{99m}TcO_4^-$	TLC-SG	Sodium citrate buffer 0.1 N pH 5.0	1
$^{99m}TcO_2$			0
^{99m}Tc-coligand			1
^{99m}Tc-HYNIC-TOC			0
$^{99m}TcO_4^-$	TLC-SG	Methylethylketone	1
$^{99m}TcO_2$			0
^{99m}Tc-coligand			0
^{99m}Tc-HYNIC-TOC			0
$^{99m}TcO_4^-$	ITLC-SG	Methanol:ammonium acetate (1:1)	1
$^{99m}TcO_2$			0
^{99m}Tc-coligand			1
^{99m}Tc-HYNIC-TOC			1

The percentage of labeled peptide (^{99m}Tc-HYNIC-TOC) as well as the percentage of radiochemical impurities were determined in TLC systems, considering the sum of segments in the chromatographic strips as showed in Fig. 2.

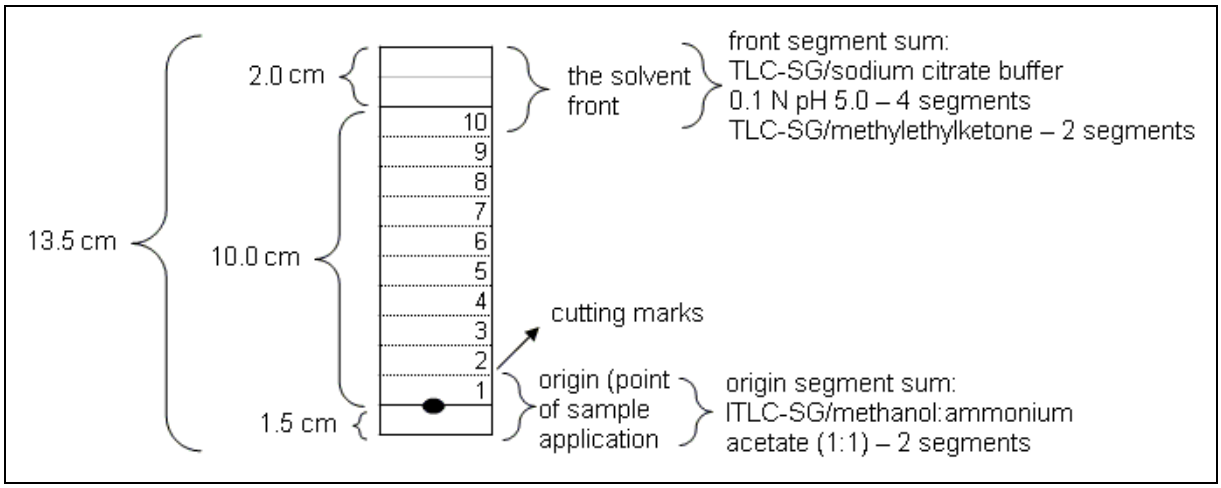

Figure 2. Chromatographic strip representation utilized in radiochemical purity control with cutting marks

All experiments were performed in duplicate, it means, two chromatographic strips to each mobile phase.

In order to check the stability of the preparation, radiochemical purity of the labeling mixtures were determined (N= 6), 30 minutes and 5 hours after labeling procedure.

3. RESULTS

The employed labeling procedure resulted in high radiochemical yield that means, the percentage of the ^{99m}Tc-HYNIC-TOC in the preparation were superior than 90 %, when determined by TLC.

HPLC chromatograms did not show any changes in radiochemical purity during the time course considered for the analysis and the values were higher than 90 %. Retention time of ^{99m}Tc-HYNIC-TOC was 13.17 minutes, while free ^{99m}Tc was eluted at 3.25 minutes (Fig. 3).

The HPLC system also identified the ^{99m}Tc-coligand, a radiochemical impurity that can be identified in the preparation (RT= 4.57) (Fig. 3).

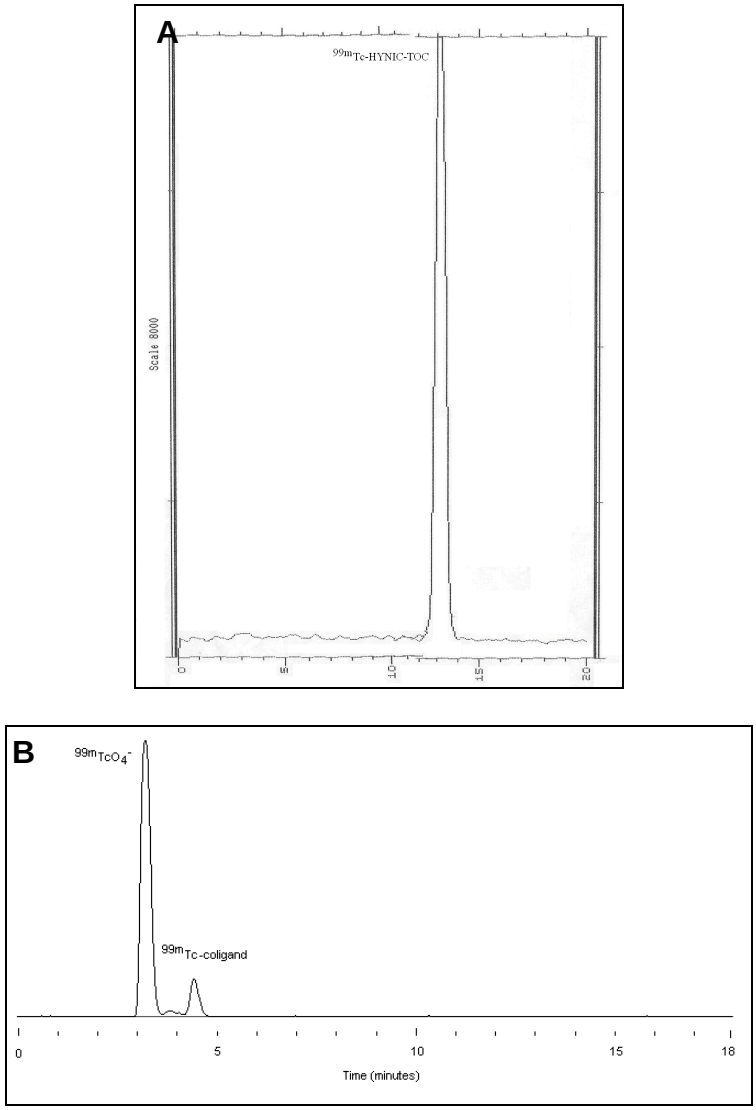

Figure 3. HPLC chromatograms of (A) ^{99m}Tc-HYNIC-TOC, (B) $^{99m}TcO_4^-$ and ^{99m}Tc-coligand

Table 2 represents the percentage of the ^{99m}Tc-HYNIC-TOC in five preparations, 30 minutes and 5 hours after labeling procedure.

Table 2. Radiochemical yield distribution of each labeling procedure

Labeling procedure	% ^{99m}Tc-HYNIC-TOC	
	30 min	5 h
1	94.20 ± 0.1	93.92 ± 0.1
2	94.86 ± 0.1	96.28 ± 0.1
3	90.88 ± 3.61	93.71 ± 1.35
4	90.94 ± 1.06	92.26 ± 0.20

Labeling procedure	% ^{99m}Tc-HYNIC-TOC	
	30 min	5 h
5	92.01 ± 0.99	90.66 ± 0.52
Average	93.58 ± 1.85	93.36 ± 2.09

The high stability of labeled peptide can be also evidenced on Fig. 4.

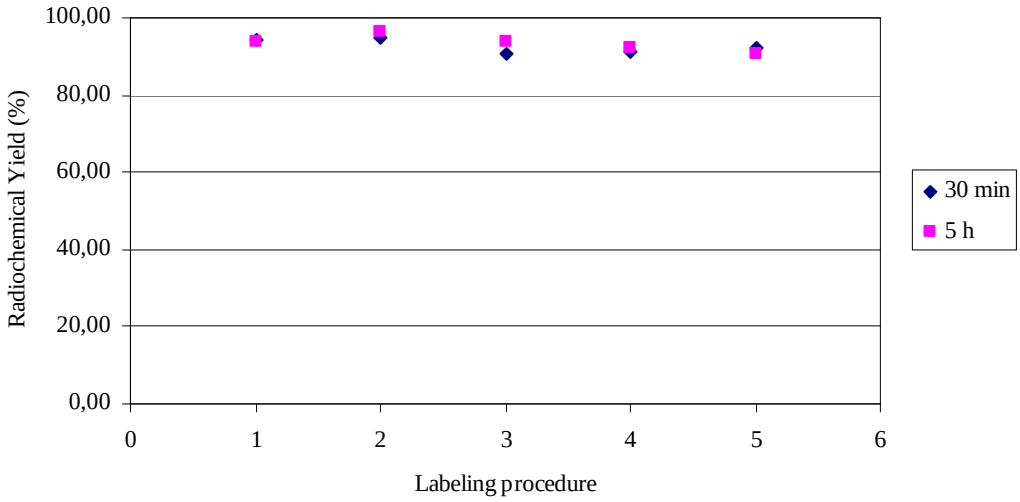

Figure 4. Radiochemical yield distribution of 5 radiolabeling sessions

The mean percentage of the different radiochemical impurities presented in the five preparations can be evidenced on Table 3

Table 3. Radiochemical yield distribution of 5 preparations

N° of labeling	Average yield (%)			Range of yield (%)	
	Radiochemical species	30 min	5 hours	30 min	5 hours
5	$^{99m}TcO_4^-$	0.54 ± 0.40	3.45 ± 4.11		
	$^{99m}TcO_4^-$ + ^{99m}Tc-coligand	7.29 ± 3.62	7.92 ± 5.42		
	^{99m}Tc-coligand	6.75 ± 3.31	4.48 ± 1.81		
	$^{99m}TcO_2$	1.54 ± 1.22	0.82 ± 0.49		
	^{99m}Tc-HYNIC-TOC	91.17 ± 3.83	91.25 ± 5.50	90.88 – 94.86	90.66 – 96.28

The pH value of the product, measured by pH strip, was about 6.5.

4. DISCUSSION

The proposed method for the labeling of HYNIC-TOC with ^{99m}Tc was demonstrated to be reliable and reproducible, with high radiochemical yield and low cost in the routine practice, especially if compared with ^{111}In-DTPA-octreotide that is now used in routine procedures for diagnostic and staging of neuroendocrine tumors.

The good radiochemical yield and stability of the peptide labeled with technetium stimulate future works for development of a lyophilized reagent that enable routine utilization.

Radio HPLC and TLC-SG methods had shown comparable results: radiochemical purity was higher than 90 % and the product remained stable during 5 hours.

Because TLC is an easier and faster protocol widely used in the nuclear medicine department, the HPLC validation described in this paper allows the use of this approach for routine application in the quality control of this radiopharmaceutical.

5. CONCLUSIONS

We can conclude that ^{99m}Tc- HYNIC-TOC represents a reliable tool for routine clinical application in the diagnostic of neuroendocrine tumors instead of ^{111}In labeled peptide.

REFERENCES

1. E. R. Obenaus, J. L. Crudo, M. M. Edreira, S. G. Castiglia, “Analogos de somatostatina marcados con ^{99m}Tc,” *Ver. Med. Nucl. Alasbimn J.*, Santiago, http://www.alasbimnjournal.cl/19obenaus.html (2003).
2. Q. Wang, K. Graham, T. Schauer, T. Fietz, A. Mohamed, X. Liu, J. Hoffend, U. Haberkorn, M. Eisenhut, W. Mier, “Pharmacological properties of hydrophilic and lipophilic derivatives of Octreotate,” *Nucl. Med. Biol.*, **v. 31**, pp. 21-30 (2004).
3. A. Placcińska, R. Mikolajczak, H. R. Maecke, E. Mlodkowska, J. Kunert-Radek, A. Michalski, K. Rzeszutek, J. Kozak, J. Kuśmierek, “Clinical usefulness of 99mTc-EDDA/HYNIC-TOC scintigraphy in oncological diagnostics: a preliminary communication,” *Eur. J. Nucl. Med. Mol. Imaging*, **v. 30**, n. 10, pp. 1402-1406 (2003).
4. M. Bangard, M. Béhé, S. Guhlke, R. Otte, H. Bender, H. R. Maecke, H. J. Biersak, “Detection of somatostatin receptor-positive tumours using the new 99mTc-tricine-HYNIC-D-Phe1-Tyr3-octreotide: first results in patients and comparison with 111In-DTPA-D-Phe1-octreotide,” *Eur. J. Nucl. Med.*, **v. 27**, n. 6, pp. 628-637 (2000).
5. J. B. Cwikla, R. Mikolajczak, D. Pawlak, J. R. Buscombe, A. Nasierowska-Guttmejer, A. Bator, H. R. Maecke, J. Walecki, “Initial direct comparison of ^{99m}Tc-TOC and ^{99m}Tc-

TATE in identifying sites of disease in patients with proven GEP NETs," *J. N. Med.*, **v. 49**, n. 7, pp.1060-1065 (2008).
6. J. Kosowicz, R. Mikolajczak, R. Czepczyński, K. Ziemnicka, M. Gryczyńska, J. Sowiński, "Two peptide receptor ligands ^{99m}Tc-EDDA/HYNIC-Tyr^3-octreotide and ^{99m}Tc-EDDA/HYNIC-$_D$Glu-octagastrin for scintigraphy of medullary thyroid carcinoma," *Cancer Biother. Radiopharm.*, **v. 22**, n. 5, pp.613-628 (2007).
7. S. Liu, D. S. Edwards, "99mTc-labeled small peptides as diagnostic radiopharmaceuticals," *Chem. Rev.*, **v. 99**, pp. 2235-2268 (1999).
8. E. von Guggenberg, B. Sarg, H. Lindner, L. M. Alafort, S. J. Mather, R. Moncayo, C. Decristoforo, "Preparation via coligand exchange and characterization of [^{99m}Tc-EDDA-HYNIC-D-Phe^1,Tyr^3]octreotide (^{99m}Tc-EDDA/HYNIC-TOC)," *J. Label. Compd. Radiopharm.*, **v. 46**, pp. 307-318 (2003).
9. M. Gabriel, C. Decristoforo, E. Donnemiller, H. Ulmer, C. W. R. Rychlinski, S. J. Mather, R. Moncayo, "An intrapatient comparison of 99mTc-EDDA/HYNIC-TOC with 111In-DTPA-octreotide for diagnosis of somatostatin receptor-expressing tumors," *J. Nucl. Med.*, **v. 44**, n. 5, pp. 708-716 (2003).
10. E. von Guggenberg, R. Mikolajczak, B. Janota, C. Riccabona, C. Decristoforo, "Radiopharmaceutical development of a freeze-dried kit formulation for the preparation of [99mTc-EDDA-HYNIC-D-Phe1,Tyr3]-octreotide, a somatostatin analog for tumor diagnosis," *J. Pharm. Sci.*, **v. 93**, n. 10, pp. 2497-2506 (2004).
11. C. Decristoforo, S. J. Mather, C. Witold, E. Donnemiller, G. Riccabona, R. Moncayo, "99mTc-EDDA/HYNIC-TOC: a new 99mTc-labelled radiopharmaceutical for imaging somatostatin receptor-positive tumors: first clinical results and intra-patient comparison with 111In-labelled octreotide derivatives," *Eur. J. Nucl. Med.*, **v. 27**, n. 9, pp. 1318-1325 (2000).