Journal of Engineering Technology and Applied Sciences, 2016 *e-ISSN:* 2548-0391
Received 11 May 2016, Accepted 12 December 2016 *Vol. 1, No. 3, 149-155*
Published online 27 December 2016 *doi: will be added*

Citation: Taşdelen, B . "Practıcal Aspects of 68Ga-Labelled Dota-Peptides for Peptide-Receptor Radionuclide Therapy". Journal of Engineering Technology and Applied Sciences 1 (3) 2016 : 149-155

PRACTICAL ASPECTS OF ^{68}GA-LABELLED DOTA-PEPTİDES FOR PEPTİDE-RECEPTOR RADİONUCLİDE THERAPY

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Abstract

^{68}Gallium-labeled receptor ligands, such as ^{68}Gallium DOTATOC, ^{68}Gallium-DOTANOC, and ^{68}Gallium-DOTATATE which are used in PET are becoming gold standard of ^{68}Ga-labeled peptides. The ^{68}Ga-labeled peptides show a rapid renal clearance and are rapidly accumulated in the tumors (80% within 30 minutes); concentration in tissues without expression of somatostatin receptors is low, providing higher contrast imaging. Here, this paper presents the practical aspects and the results of preparation and radiolabelling DOTA-peptides with TiO2-based commercially available ^{68}Ge/^{68}Ga generator by using fully automated radiopharmaceutical synthesis device. The radiochemical purity (RCP) of ^{68}Ga-labeled DOTA-peptides has been investigated using high performance liquid chromatography (HPLC) and instant thin layer chromatography (ITLC). The labeling procedure resulted in high radiochemical yield. The system allows the fully automated, efficient and rapid preparation of ^{68}Ga-DOTA-derivatized peptides.

Keywords: ^{68}Ga, DOTANOC, DOTATATE, peptides, PET

1. Introduction

^{68}Ga is a short-lived positron emitter (half-life 67.6 min), the production of which requires no cyclotron since it is available as a generator nuclide. ^{68}Ge/^{68}Ga generators (half-life of the parent radionuclide ^{68}Ge=270.8 days) are commercially available systems. ^{68}Ga has been used for medical purposes since the early 1960s [1,2] and Deutsch proposed the use of ^{68}Ga for PET in the same way as ^{99m}Tc for SPECTS [3]. ^{68}Ge/^{68}Ga radionuclide generators have been the object of development and investigation for almost fifty years. Rösch et al. made a recent review on this and other PET radionuclide generator systems [4]. Such a generator-based radionuclide, ^{68}Ga, is getting into the focus of the researchers and clinicians especially for radiolabelling of biomolecules [5]. The advent of small peptides has boosted the interest in this radionuclide as its short half life of 67.7 min is well suited to their rapid pharmacokinetics and

results in a low radiation burden for the patient. Additionally, radiolabeling with trivalent metals such as ^{68}Ga is straight forward using direct reaction with DOTA (1,4,7,10-tetraazcyclododecane-1,4,7,10-tetraacetic acid) derivatized peptide conjugates.
The most recent somatostatin analogues are DOTANOC [1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid]-1-Na13-ocreotide], DOTATATE (DOTA-Tyr^3-Octreotate) and DOTA-TOC [DOTA-D-Phe^1-Tyr^3-ocreotide] [5-7].

So far, a variety of methods has been described for high efficiency labeling of DOTA conjugated peptides based on manually, semi-automated or fully automated systems [8-14]. This manuscript presents radiochemical purity assessment of [^{68}Ga]-DOTA-peptides with TiO_2-based commercially available ^{68}Ge/^{68}Ga generator by using fully automated radiopharmaceutical synthesis device with preconcentrated and purified generator eluate using a cation exchange-cartridge.

2. Materials and methods

2.1. Materials

The ^{68}Ge/^{68}Ga Generator was obtained from Ecker & Ziegler Eurotope (Berlin, Germany) with the activity of 1480 MBq (40 mCi). For full automation a PC-controlled radiopharmaceutical synthesis device based on modular concept (Modular Lab. Eckert & Ziegler Eurotope, Berlin Germany) was used for all steps in the synthesis. The ^{68}Ge/^{68}Ga Generator was eluted with 0.1 M HCl.

Acetone, methanol and ammonium acetate were obtained from Merck. DOTA-derivatised peptides (DOTANOC, DOTATATE) were obtained from PiCHEM (Graz, Austria). A fraction of the ^{68}Ge/^{68}Ga Generator (40 mCi, Eckert& Ziegler Eurotope, Berlin, Germany) eluate was added to a solution containing the DOTA-Tyr3-octreotate (DOTATATE) or DOTANOC in sodium acetate buffer solution.

Radiolabelling was performed as described by Breeman et al [15]. Briefly a fraction of the generator eluate was added to one-tenth of the eluate volume of a solution containing 20 μg of DOTATATE or DOTANOC in 1.25 mol.L^{-1} sodium acetate solution. The solution was heated at 95^{o}C for 7 min (3 min preheating) and transferred to a preconditioned C-18 reversed phase cartridge (SEP PAK Mini Waters) for purification. The cartridge was eluted with ethanol over sterile filter (Millex-GV, Millipore) into the final sterile vial. Subsequently, cartridge and sterile filter washed with saline to dilute final ethanol content. The whole process from elution of the generator to the final product was performed within 12 min and the whole process was performed with fully automated PC-controlled radiopharmaceutical synthesis device based on a modular concept.

2.2. Analysis

2.2.1. Thin-layer chromatography

Method 1: 0.1 M Na-citrate buffer pH 5/ ITLC-SG; R_F (free ^{68}Ga)=0.8-1.0, R_F (^{68}Ga peptide)=0.0-0.3. Method 2: 1 M ammonium acetate/methanol (1:1); R_F (^{68}Ga colloid)=0-0.2, R_F (^{68}Ga peptide)=0.8-1.0.

2.2.2. High-performance liquid chromatography

The HPLC system consists of Agilent quaternary pump with degasser; 4.6 mm (i.d.) x 250 mm C-18 5-µm column; Agilent Variable UV detector; GabiStar gamma flow monitor with GINA star program (Raytest). The flow rate was maintained at 1 mL/min. The mobile phase consisted of 0.1% trifluoroacetic acid (TFA) in water /acetonitrile (ACN): t:0-3 min 0%ACN, 3-10 min 0-40%ACN, 10-20 min 40%ACN, 20-23 min 40-70%ACN, 26-27 min 70-0%ACN, 27-30 min 0%ACN. Samples were analyzed using a 20 µL injection.

3. Results and discussion

First, an accurate and simple high-performance liquid chromatographic (HPLC) assay was performed for the HPLC analysis of DOTANOC and DOTATATE. The HPLC system comprises a C-18 column and the detection is performed by UV absorbance measurements at 254 nm. Retention times for DOTANOC and DOTATATE were 13.7 and 12.5 min, respectively (Fig. 1a and b).

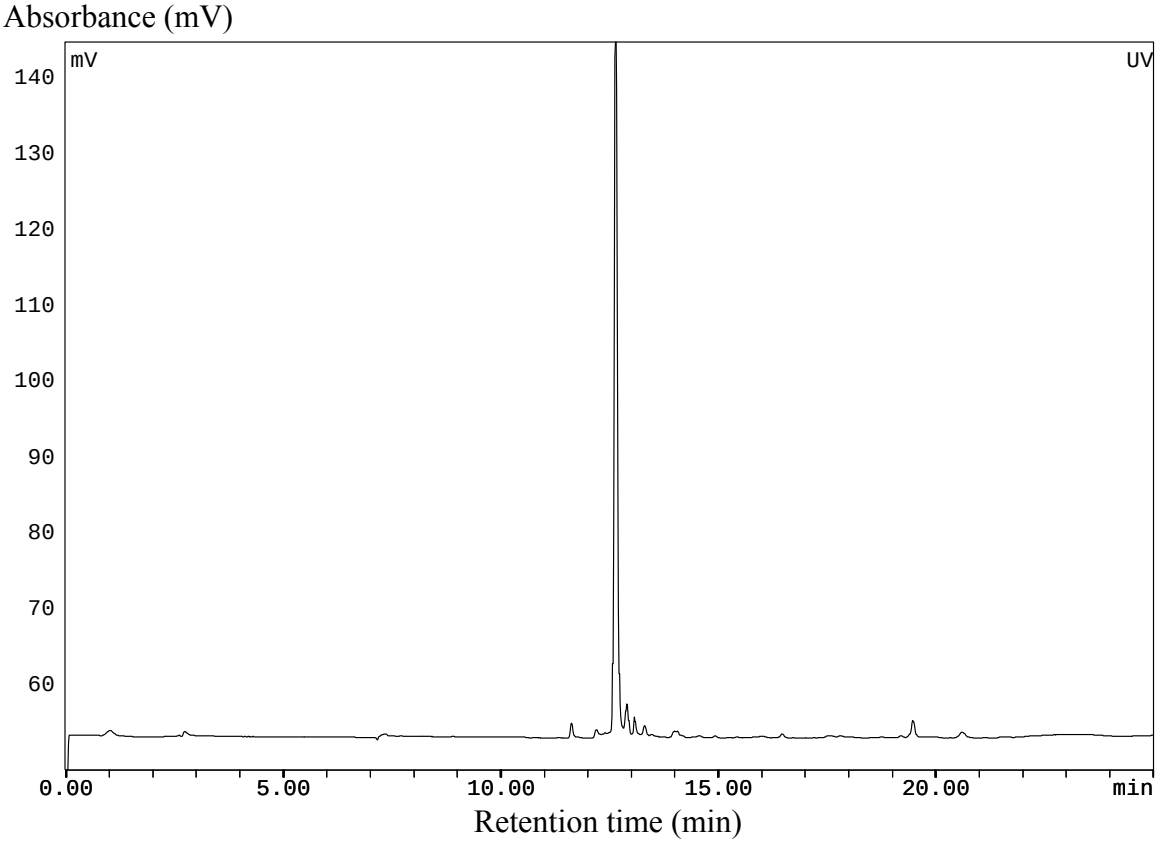

Figure 1a. HPLC chromatogram of DOTATATE

ITLC systems enabled the separation of radiochemical species presented in the labeled mixture. In ITLC, two different solvent systems were used. 0.1 M sodium citrate pH=5 was used to determine the amount of free Ga (R_f =0.8-1). Finally, methanol/1 M ammonium acetate 1/1 solvent system was used to determine Ga-colloid (R_f=0) (Table 1).

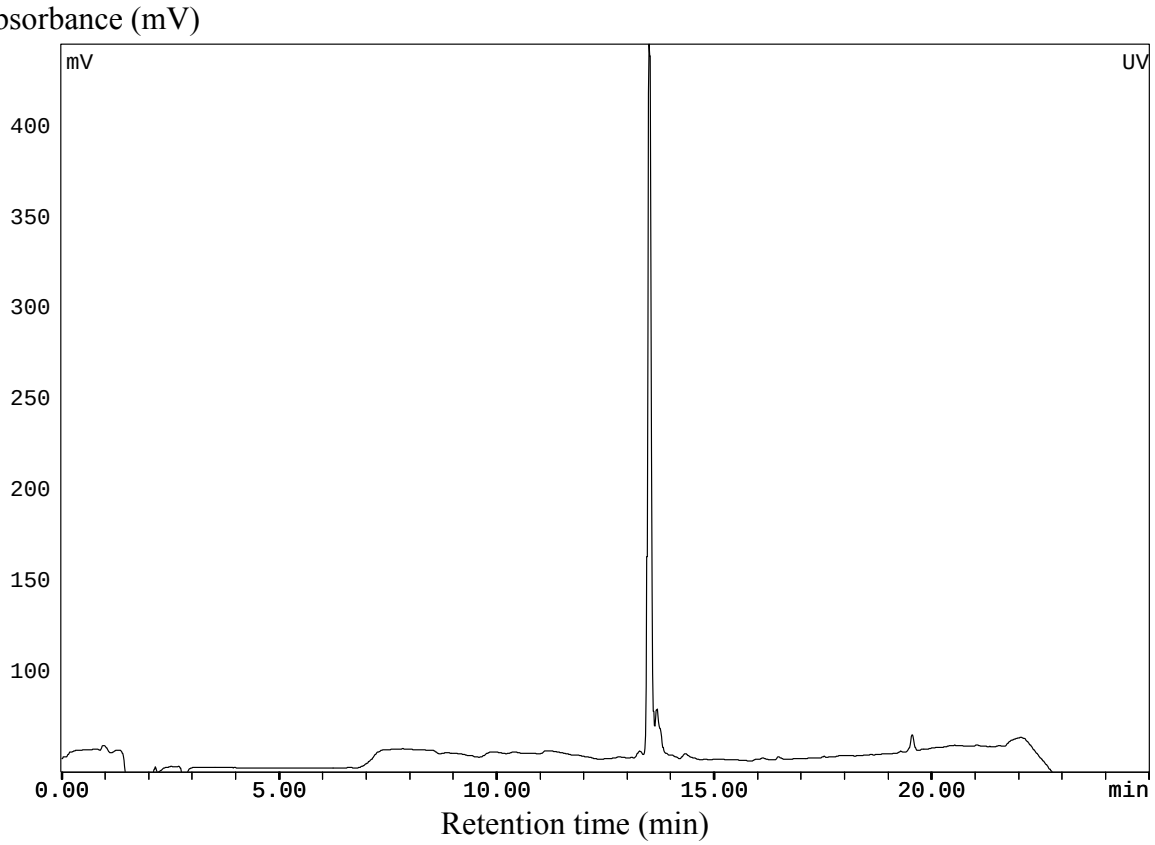

Figure 1b. HPLC chromatogram of DOTANOC

After preparation of ^{68}Ga-DOTANOC, RCP result from ITLC is 94.8%. Main impurity came from ^{68}Ga-colloid. After SEPPAK purification of ^{68}Ga-DOTATATE, radiochemical purity (RCP) increased from 94.8% to 99.1% due to removal of colloidal ^{68}Ga. In the same way, RCP for ^{68}Ga- DOTANOC increased from 95.3% to 99.2%.

RCP was also found to be >98.0% by using HPLC after SEPPAK purification of ^{68}Ga-DOTATATE and ^{68}Ga-DOTANOC, Fig. 2a and Fig. 2b show the HPLC chromatograms of ^{68}Ga- DOTATATE and ^{68}Ga-DOTANOC, respectively. We see that the retention times for ^{68}Ga-DOTATATE and ^{68}Ga-DOTANOC were 12.5 and 13.7 min, respectively. Ocak et al. has pointed that during HPLC analysis of the SEPPAK-purified labeled peptides, a small peak elution prior to ^{68}Ga-DOTATOC became more prominent when using small amounts of peptide [14]. They used more than 20 μg of the peptide in order to avoid considerable formation of this peak. However, in our work, 20 μg of the peptide had been used since we had limited supply of the peptides. Therefore, a small peak eluates prior to ^{68}Ga- DOTATATE or ^{68}Ga-DOTANOC as mentioned in [14].

Table 1. Solvent systems used in ITLC and R_f values of the radiochemical species.

Solvent	^{68}Ga-DOTATATE/DOTANOC	Free Ga	Ga-colloid
0.1 M Citrate pH 5	0	0.8-1	0

Methanol/Ammonium acetate 1/1	0.8-1	0	0

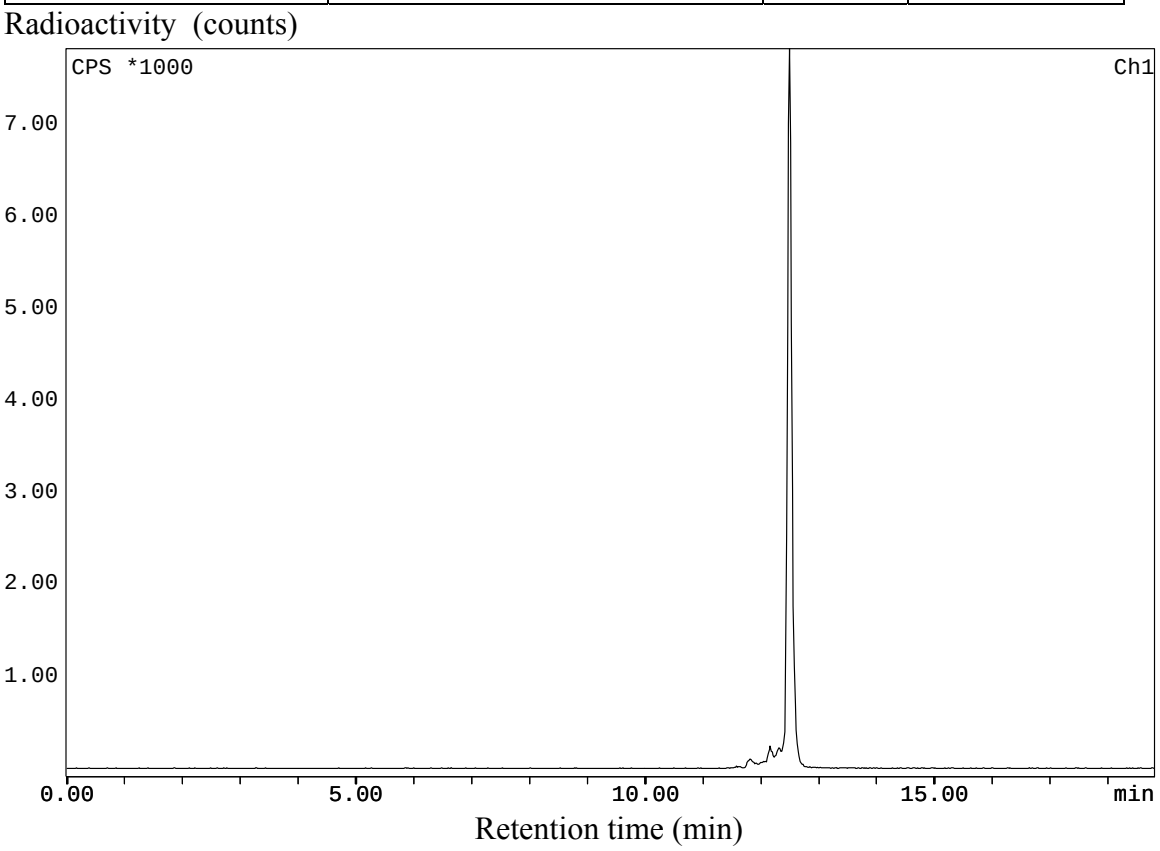

Figure 2a. HPLC Chromatogram of ^{68}Ga- DOTATATE

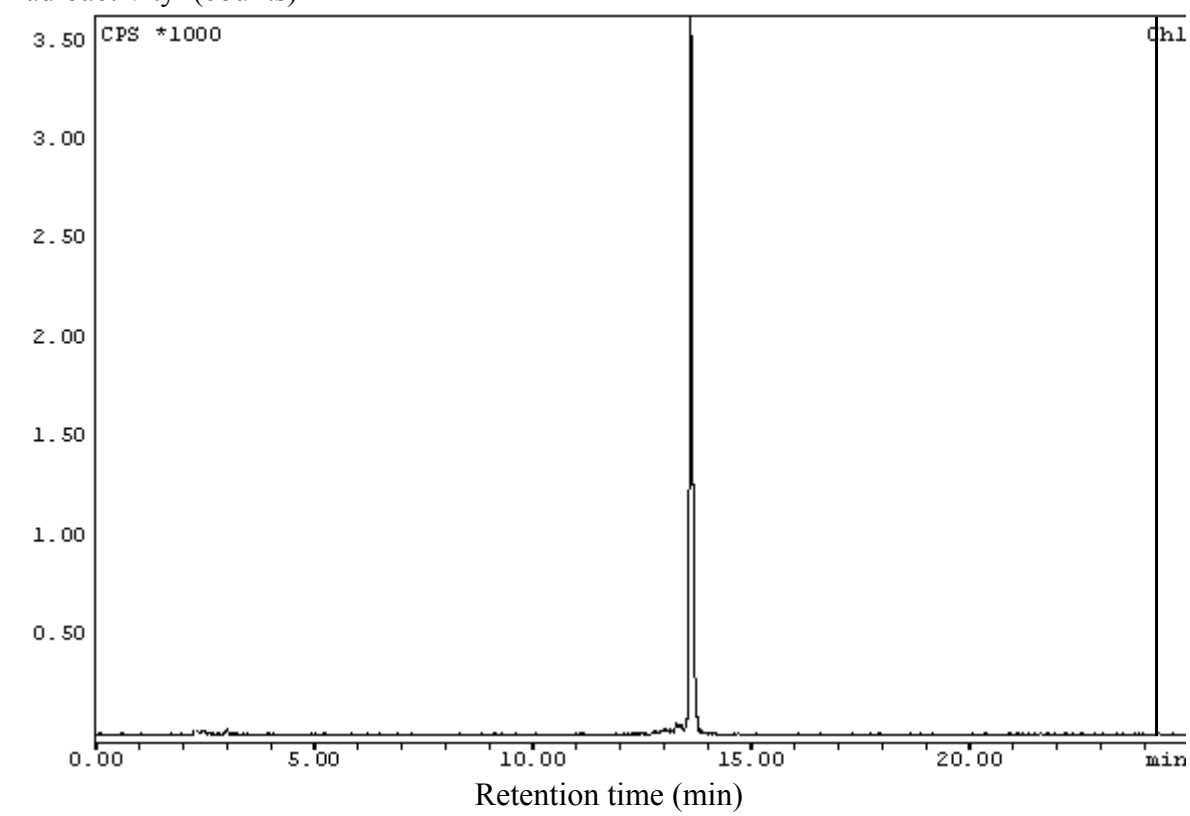

Figure 2b. HPLC Chromatogram of ^{68}Ga-DOTANOC

3. Conclusion

Radio-HPLC and ITLC methods had shown comparable results: radiochemical purity was always higher than 99% after SEPPAK purification of ^{68}Ga-labelled DOTA-derivatized peptides. The described system allows the fully automated, successfully and reproducibly preparation of ^{68}Ga-DOTATATE or ^{68}Ga- DOTANOC with reduced radiation dose to the operator. It can be concluded that this system provide a reproducible, high throughput and reliable tool for routine clinical practices.

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