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Single vial formulation for theranostic radiopharmaceutical preparation

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Abstract The present work was aimed at development of pharmaceutical grade single vial kit like formulation of somatostatin analogue, DOTA–Tyr^{3}–Thr^{8}-Octreotide (DOTATATE) suitable for radiolabeling with both diagnostic (^{68}Ga) and therapeutic (^{177}Lu) radioisotope. Single vial kit like formulation of DOTATATE was prepared. Radiolabeling methods with ^{68}Ga and ^{177}Lu were standardized. The pharmaceutical purity and stability of formulation was studied over a period of 6 months. Pharmacokinetics of radiolabeled preparations was studied in Swiss mice. DOTATATE formulation with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer was successfully prepared. Both ^{68}Ga–DOTATATE and ^{177}Lu–DOTATATE complexes were formed with >95 % radiochemical purity. Biodistribution studies of ^{68}Ga–DOTATATE and ^{177}Lu–DOTATATE complexes in Swiss mice revealed fast clearance of activity via renal route. Single vial kit like formulation suitable for easy preparation of ^{68}Ga–DOTATATE and ^{177}Lu–DOTATATE at hospital radiopharmacy was successfully demonstrated.

Keywords DOTATATE formulation · ^{68}Ga–DOTATATE · ^{177}Lu–DOTATATE · Theranostic · PRRT

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Introduction

Radiolabeled somatostatin (SST) analogues, Tyr^{3}–Thr^{8}-octreotide (TATE), Tyr^{3}-octreotide (TOC) and NaI^{3}-octreotide (NOC) are successfully being utilized for early diagnosis and targeted radiotherapy of somatostatin receptor over expressing tumors [1, 2]. Macro cyclic ligand, 1,4,7,10-tetraazacyclododecane,1,4,7,10-tetra acetic acid (DOTA) forms thermodynamically stable and kinetically inert complexes with lanthanides and is an extensively used bifunctional chelating agent for radiolabeling biomolecules with ^{177}Lu and ^{90}Y radioisotopes. DOTA also forms stable complex with ^{68}Ga (III). Although other ligands such as NOTA, PCTA are proposed to be better for ^{68}Ga labeling, DOTA conjugated SST and RGD analogues have been found to be useful in clinics. Hence, DOTA conjugates of biomolecules have the advantage of being developed as theranostic radiopharmaceutical [3]. Envisaging the need for easy labeling methods and availability of ^{68}Ga and ^{177}Lu radiopharmaceuticals at a low cost for clinical applications, a pharmaceutical grade kit formulation of DOTA peptide suitable for radiolabeling with both diagnostic (^{68}Ga) and therapeutic (^{177}Lu) radioisotopes was attempted. Radiolabeling of DOTA peptides with radioisotopes is generally reported using ammonium acetate or sodium acetate buffer at pH 4–4.5. Although, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer is superior to acetate buffer that is

currently used in preparation of ^{68}Ga, however its use for ^{90}Y and ^{177}Lu labeled peptide radiopharmaceuticals, is not reported [4]. Herein, we report the standardization of a single vial kit formulation of DOTATATE using HEPES, radiolabeling with ^{177}Lu and ^{68}Ga from an indigenously assembled semi automated module, evaluation of stability of the formulation for a period of 6 months and biological evaluation of radiopharmaceuticals in Swiss mice.

Experimental

DOTATATE was obtained from piChem, Austria and HEPES was procured from Sigma Chemical Company, USA. Sep-Pak C18 cartridges (1 cc) from Waters India Ltd and Strata X-C column (1 cc) from Phenomenex, USA were procured. ITLC-SG paper from Agilent Technologies USA was used for characterization of the complex by ITLC. 3 mm Whatman chromatography paper was used for paper chromatography. 0.22 μ membrane filter (33 mm) was obtained from Millipore Corporation, USA. Other chemicals and solvents were of AR grade, procured from reputed manufacturers from India.

Pharmaceutical grade $^{177}LuCl_3$ with specific activity >20 Ci/mg was produced locally at Isotope Applications and Radiopharmaceutical Division, BARC. ^{68}Ga generator was obtained from iThemba, South Africa. All radioactivity measurements were made using NaI (Tl) scintillation counter. The high performance liquid chromatography (HPLC) system used was obtained from JASCO (PU 1580), Japan, equipped with a PU 1575 UV/VIS detector. A well-type NaI (Tl) scintillation detector was coupled to the system for radioactivity measurements. All the solvents used for HPLC analyses were of HPLC grade and purchased from reputed local manufacturers, degassed and filtered prior to use.

Alpha 1–2 LD plus freeze dryer was from Martin Christ, GmBH. Sterility test kit containing fluid thioglycolate media and soybean casein media was obtained from Himedia Laboratories, India and Endosafe PTS equipment and cartridges (sensitivity: 0.5–0.05 EU/mL) for endotoxin detection were procured from Charles River Laboratories India Pvt. Ltd.

DOTA peptide formulation

All glassware used for ^{68}Ga labeling was acid washed. The formulation was carried out under laminar flow of class 100 set up in an in-house modular clean room facility maintained and validated as per standard procedures. Kit formulations of DOTATATE using HEPES buffer in a single vial was standardized. Stock solution was prepared by adding 1 mg/mL DOTATATE to 0.5 M HEPES buffer, pH 4.0 and mixed. The mixture was filter sterilized under aseptic conditions. The formulation corresponding to 30, 50 and 200 μg DOTATATE (20 vials each) in HEPES was aseptically dispensed in sterile glass vials of 10 mL capacity, freeze dried and sealed under vacuum after lyophilization. The formulations were stored at −20 °C and tested for radiolabeling at periodic intervals. Sterility test was carried out by the standard procedures. The kits were reconstituted in 1 mL sterile saline and the sample was incubated in Soybean casein digest medium at 20–25 °C for 14 days and in Fluid thioglycollate medium at 30–35 °C for 14 days. The observations made were compared with positive and negative controls set up for each test. BET test was carried out by using Endosafe PTS system after appropriate dilution of the samples and loading 25 μL sample on cartridges (sensitivity 0.5–0.05 EU/mL).

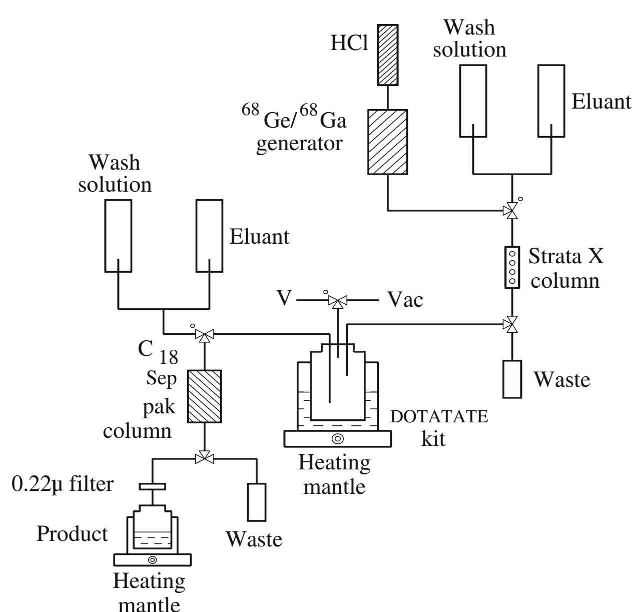


Fig. 1 Schematics of semi-automated module for ^{68}Ga–DOTATATE preparation

Radiolabeling of formulation with ^{68}Ga

^{68}Ga elution and radiolabeling

Indigenously assembled semi automated module was setup for ^{68}Ga elution, radiolabeling and purification of ^{68}Ga–DOTATATE (Fig. 1). ^{68}Ga eluted in 5 mL of 0.1 M HCl from the ^{68}Ge/^{68}Ga generator was loaded on Strata X-C column. To remove metal ion impurities, the column was washed with acetone/0.1 N HCl (80/20) and purified ^{68}Ga was eluted in 0.5 mL of acetone/0.02 N HCl (97.6/2.4). The purified ^{68}Ga (37–185 MBq) was directly added to the 30 and 50 μg kit vial reconstituted in 0.5 mL of sterile

ultrapure water. Radiolabeling was carried out by heating at 90 °C for 10 min.

Purification

To remove the HEPES from the final preparation and colloidal species if any, complex was purified by Sep-Pak C18 cartridge. Cartridge (1 cc capacity) was preconditioned with methanol, the reaction mixture containing ^{68}Ga–DOTATATE was diluted with water and loaded on the column. The complex was retained on the cartridge, which was washed with water to remove any impurities. The purified product was eluted with 0.5 mL ethanol through a 0.22 µ membrane filter. The ethanol was removed by heating at 80 °C as shown on Fig. 1 and the product was reconstituted in sterile saline.

Characterization

^{68}Ga–DOTATATE complex was characterized by HPLC using gradient elution with Solvent A: H_2O with 0.1 % TFA, Solvent B: acetonitrile (ACN) with 0.1 % TFA (Gradient: 0–4 min: 5 % B, 4–20 min: 5–95 % B, 20–30 min: 95–5 % B, radioactivity detector 400–600 keV). ITLC was carried out using two solvent systems. In acetonitrile: methanol (1:1 v/v) solvent, the complex migrated to the solvent front while in 0.1 N citrate buffer, complex remained at the origin. Paper chromatography (PC) in 50 % acetonitrile wherein complex migrates along with solvent was also carried out to estimate the radiochemical yield.

Stability studies

Stability of the complex, 2 and 4 h post preparation was evaluated by HPLC. 50 µL of the complex was incubated with 500 µL of human serum from healthy volunteer for 1 h at 37 °C to test serum stability. The sample was precipitated with acetonitrile and centrifuged at 3,000 rpm for 5 min. Supernatant obtained after precipitation was used for PC, ITLC and HPLC analysis.

Radiolabeling of formulation with ^{177}Lu

The formulation was tested for labeling with ^{177}Lu 1.1, 1.85 and 7.4 GBq of ^{177}Lu was added to the 30, 50 and 200 µg DOTATATE formulation vials respectively and incubated at 95 °C for 25 min. The ^{177}Lu–DOTATATE complex was purified through C18 column for removing HEPES using semi-automated setup similar to that used for ^{68}Ga–DOTATATE preparation and filtered through 0.22 µm filter. The complex was characterized using HPLC and paper chromatography in 50 % aqueous acetonitrile. 50 mg of sterile ascorbic acid solution was added to the complex. Stability of the complex in saline and serum was also studied.

Stability of formulation

Stability of kit formulation was studied for a period of 6 months by carrying out radiolabeling with ^{68}Ga and ^{177}Lu and characterization by PC, ITLC and HPLC at regular intervals.

Bioevaluation studies

All animal experiments were carried out as per guidelines, regulations and approval from local animal ethics committee.

Biodistribution studies

Biodistribution studies of ^{68}Ga–DOTATATE and ^{177}Lu–DOTATATE were carried out in normal Swiss mice. ^{68}Ga–DOTATATE and ^{177}Lu–DOTATATE preparations were diluted in saline and ~0.1 mL (3–4 MBq) of the complex was injected via tail vein into separate set of mice. The animals injected with ^{68}Ga–DOTATATE were sacrificed at the end of 1 h. Mice injected with ^{177}Lu–DOTATATE were sacrificed at 1, 3 and 24 h post-injection. Four animals were used for each time point. The tissues and the organs were excised and the radioactivity associated with organs/tissues was measured in a flat type NaI (Tl) scintillation counter. The distribution of the activity in different organs was calculated as percentage of injected activity per g.

Results

DOTA peptide formulation

Single vial kit formulation containing varying amounts (30–200 µg) DOTATATE were successfully prepared using HEPES buffer. Formulations were tested and found to be of pharmaceutical grade as per the sterility tests. Bacterial endotoxins were <10 EU/mL, well within the limits of endotoxins specified in pharmacopoeia for clinical grade radiopharmaceuticals.

Radiolabeling of formulation with ^{68}Ga

^{68}Ga elution and radiolabeling

Figure 1 shows a schematic diagram of the semi-automatic module for ^{68}Ga–DOTATATE preparation. Radiolabeling yield calculated by paper chromatography in 50 % acetonitrile was comparable with yield calculated by using two solvent systems namely acetonitrile: methanol (1:1v/v)

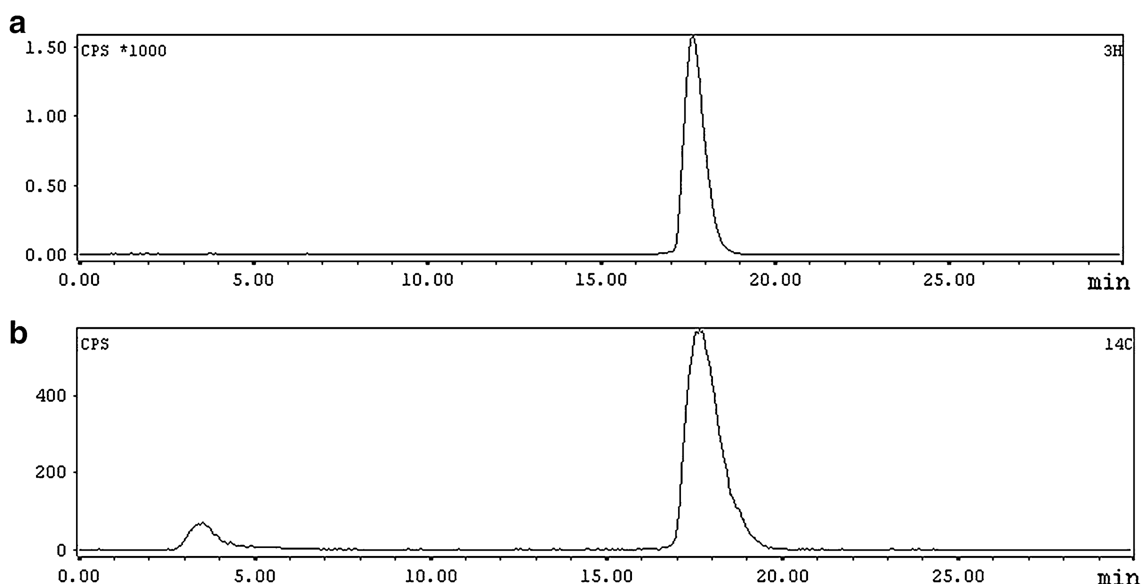


Fig. 2 **a** HPLC radiochromatogram of ^{68}Ga–DOTATATE complex. **b** Serum stability of ^{68}Ga–DOTATATE complex

solvent and 0.1 N citrate buffer and was consistently >95 %. Faster migration of solvent was observed in case of PC in 50 % acetonitrile that is advantageous for quality control analysis of ^{68}Ga-radiopharmaceuticals. Figure 2a depicts HPLC radiochromatogram of ^{68}Ga–DOTATATE complex with >99 % RCP at Rt of 17–19.5 min. ^{68}Ga–DOTA has Rt of 3–4 min in the same method.

Purification and stability of complex

^{68}Ga–DOTATATE complex was found to be stable in serum with radiochemical purity (RCP) of >90 % (Fig. 2b). The complex was stable when studied up to 4 h post preparation. Sep-Pak purification could remove impurities such as HEPES. The predominant UV peak in HPLC chromatogram due to presence of HEPES (Fig. 3a) becomes insignificant after purification (Fig. 3b).

Radiolabeling of formulation with ^{177}Lu

^{177}Lu–DOTATATE (1.1 GBq/30 μg, 1.85 GBq/50 μg and 7.4 GBq/200 μg) was prepared with >99 % RCP. Figure 4a depicts HPLC radio chromatogram of diluted sample of (200 mCi/200 μg) ^{177}Lu–DOTATATE complex with Rt of 17–20 min. Complex was stable when studied up to 7 days. ^{177}Lu–DOTATATE complex was also stable in serum as depicted in Fig. 4b.

Stability of formulation

^{68}Ga–DOTATATE and ^{177}Lu–DOTATATE with RCP > 99 % as per HPLC could be prepared when 50 μg kit

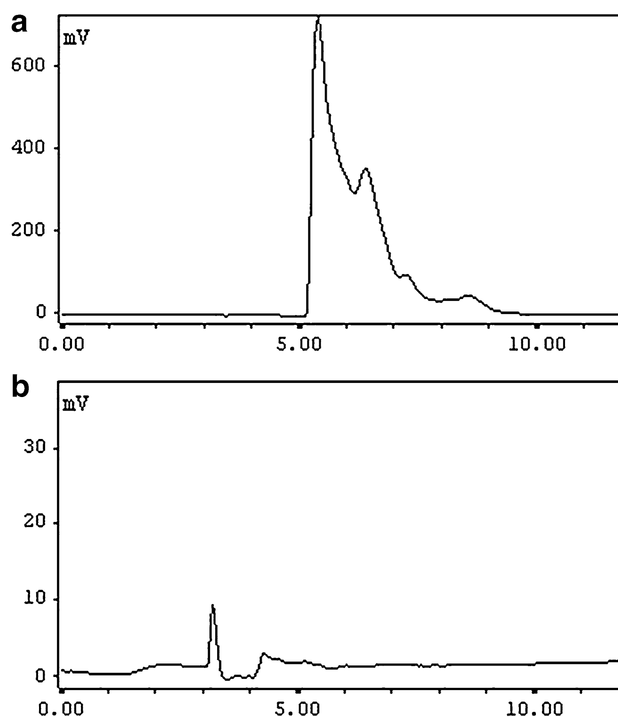


Fig. 3 **a** HPLC chromatogram of ^{68}Ga–DOTATATE complex using UV detector. **b** HPLC chromatogram of ^{68}Ga–DOTATATE complex using UV detector after Sep-Pak C18 purification

formulation was tested for stability up to 6 months as depicted in Fig. 5. HEPES buffer was effective in maintaining the desired pH for radiolabeling and hence, the formulation is expected to be useful for radiolabeling with ^{68}Ga eluted from various generators in varying eluting solvents as well as with ^{177}Lu available from various suppliers.

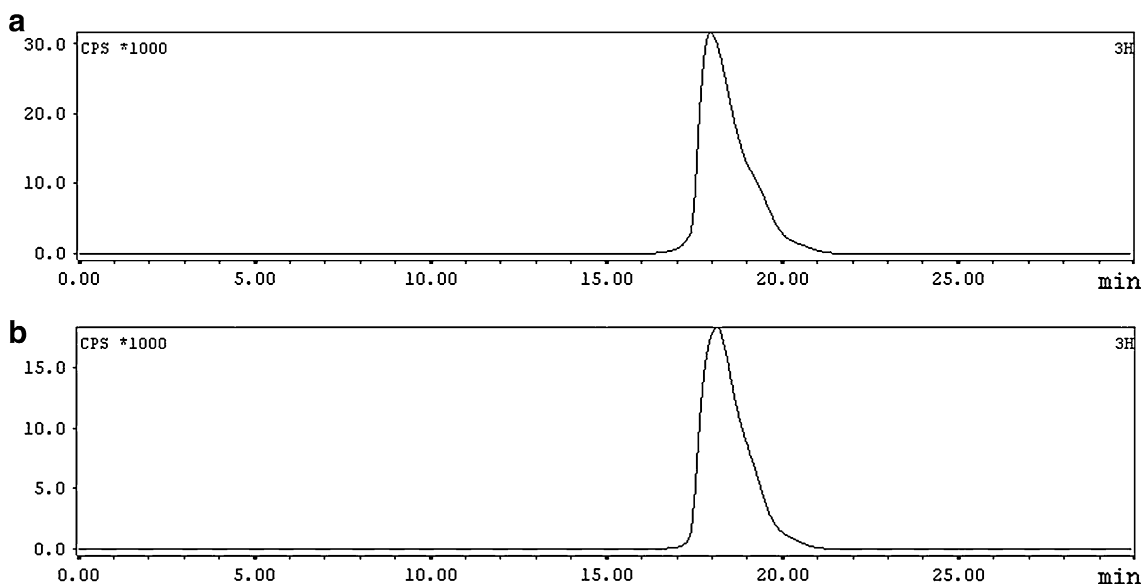
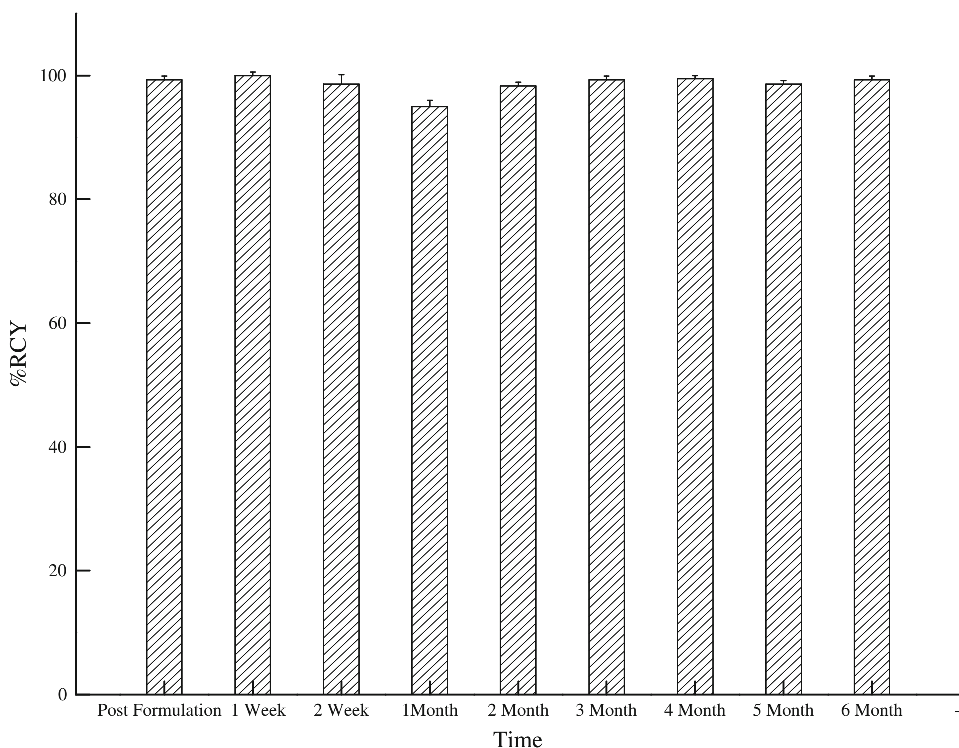


Fig. 4 **a** HPLC radiochromatogram of ^{177}Lu–DOTATATE complex. **b** Serum stability of ^{177}Lu–DOTATATE complex

Fig. 5 Radiochemical yields of ^{177}Lu–DOTATATE studied over a period of 6 months



Bioevaluation studies

Rapid clearance of ^{68}Ga–DOTATATE activity in 1 h via renal route was observed, which is expected due to hydrophilic nature of DOTA conjugate. Biodistribution studies with ^{177}Lu–DOTATATE also revealed clearance of activity via renal route with no soft tissue retention of radioactivity. Biodistribution data of ^{68}Ga–DOTATATE and ^{177}Lu–DOTATATE in Swiss mice is depicted in Table 1.

Discussion

Clinical applications of ^{68}Ga radiopharmaceuticals are expanding and showing impact in molecular imaging via clinical positron emission tomography (PET) [5]. This has also resulted in selecting patients for peptide receptor radiotherapy with ^{177}Lu–DOTATATE and ^{90}Y–DOTA-TATE. Hence, a single vial kit like DOTA peptide formulation for radiolabeling with both diagnostic and therapeutic radionuclides is desired for detection and

Table 1 Biodistribution data of ^{68}Ga–DOTATATE and ^{177}Lu–DOTATATE complex (%ID/gm) in Swiss mice

Organ/tissues	^{68}Ga–DOTATATE	^{177}Lu–DOTATATE		
	1 h	1 h	3 h	24 h
Blood	0.37 (0.14)	0.58 (0.35)	0.02 (0.02	0.03 (0.02)
Liver	0.39 (0.09)	0.50 (0.31)	0.29 (0.04)	0.18 (0.09)
Int + GB	0.89 (0.23)	1.83 (0.95)	1.51 (0.26)	0.34 (0.24)
Stomach	0.68 (0.32)	2.89 (2.00)	1.85 (0.60)	0.37 (0.44)
Heart	0.26 (0.26)	0.59 (0.37)	0.12 (0.09)	0.04 (0.08)
Lungs	1.29 (0.43)	3.55 (1.33)	3.26 (2.76)	0
Spleen	0.25 (0.32)	1.30 (0.36)	0.41 (0.20)	0
bone	0.20 (0.41)	1.5 (0.32)	0.40 (0.49)	0
Muscle	0.27 (0.29)	0.36 (0.23)	0.09 (0.09)	0
Kidneys	8.40 (2.11)	10.09 (3.53)	10.25 (5.10)	3.06 (2.62)
Excreta	86.22 (5.67)	78.38 (8.95)	85.24 (4.64)	98.24 (1.09)

Figures in the parenthesis represent standard deviations ($n = 4$)

therapy of neuroendocrine tumors (NET). Efforts were made to make a single vial formulation suitable for radiolabeling with ^{68}Ga as well as ^{177}Lu. The added advantage to this approach is if the diagnostic scan is positive, similar formulation can be prepared with therapeutic radionuclide such as ^{177}Lu for peptide receptor radionuclide therapy (PRRT).

Development of kits for small chelator coupled peptides by freeze drying was proposed by Maecke et al. [6]. Although, kit type preparation to form ^{68}Ga based radiopharmaceuticals using chelators like NOTA and NODAGA is reported at room temperature within 10 min, development of such agents and use in clinical studies is not common as differences in chelators may also influence the pharmacokinetics, affinity and tumor uptake of the agents. In clinical situations where DOTA conjugated peptides like TATE are already used, kit formulations for such agents is highly desired for easy preparation of radiopharmaceuticals at low cost [7]. Preparation of lyophilized DOTA peptide formulations, radiolabeling with ^{68}Ga and ^{177}Lu and stability studies of each formulation reported here will be useful for translating to hospital radiopharmacy.

Kit formulation in ammonium acetate buffer is not a viable option due to loss of buffer components like ammonia during lyophilization. Sodium acetate buffer was not able to maintain pH 4 after lyophilization when ^{68}Ga in 0.1 N HCl is used for complexation. Our studies revealed HEPES as a better alternative for radiolabeling with ^{68}Ga eluted from different generators and same formulation is promising for preparation of ^{177}Lu–DOTATATE. Our results show that HEPES and other impurities in the radiopharmaceutical preparation can be easily removed by solid phase extraction column purification.

Generator systems can be optimally utilized if kit formulations for preparation of radiopharmaceuticals are available. Initially 30 and 50 µg DOTATATE concentration was used for preparation of diagnostic radiopharmaceutical. Similar preparation with higher peptide concentration of 200 µg DOTA peptide was formulated for preparation of patient dose of ^{177}Lu–DOTATATE. It is reported that purification leads to elimination of components added to prevent radiolytic damage from the preparation. Hence, ascorbic acid was added after purification of ^{177}Lu–DOTATATE by Sep-Pak purification [8].

Theranostic DOTA peptide kit like formulation for radiolabeling will reduce the total cost of the radiopharmaceutical due to non-dependence on costly modules with limited life span and may lead to better management of patients with NET.

Conclusion

DOTA peptide kit formulation was successfully prepared and its application for radiolabeling with diagnostic and therapeutic radionuclides was demonstrated. Deployment of formulation for preparation of current peptide based radiopharmaceuticals is expected to expand the clinical applications of newly developed molecular imaging agents.

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References

1. Teunissen JJM, Kwekkeboom DJ, Valkema R, Krenning EP (2011) Endocr Relat Cancer 18:S27–S51
2. Chakraborty S, Vimalnath KV, Lohar S, Shetty P, Dash A (2014) J Radioanal Nucl Chem. doi:10.1007/s10967-014-3169-z
3. Breeman WA, de Blois E, Sze Chan H, Konijnenberg M, Kwekkeboom DJ, Krenning EP (2011) Semin Nucl Med 41:314–321
4. Brom M, Joosten L, Oyen WJG, Gotthardt M, Boerman OC (2012) EJNMMI Res 2:4
5. Mukherjee A, Pandey U, Chakravarty R, Sarma HD, Dash A (2014) Mol Imaging Biol. doi:10.1007/s11307-014-0719-2
6. Maecke HR, Hofmann M, Haberkorn U (2005) J Nucl Med 46:172S–178S
7. Das T, Bhadwal M, Banerjee S, Sarma HD, Shinto A, Kamaleshwaran KK (2014) J Radioanal Nucl Chem 299(3):1389–1398
8. Maus S, de Blois E, Schreckenberger M, Breeman WAP (2013) World J Nucl Med 12(1):84