Available online at www.sciencedirect.com
ScienceDirect
Applied Radiation and Isotopes 66 (2008) 1091–1096
Applied Radiation and Isotopes
www.elsevier.com/locate/apradiso

Technical note

Radiolabelling, quality control and radiochemical purity assessment of the Octreotide analogue ^{68}Ga DOTA NOC

D. Di Pierro[a], A. Rizzello[a], G. Cicoria[b], F. Lodi[a], M. Marengo[b], D. Pancaldi[b], S. Trespidi[a], S. Boschi[a,*]

[a]*PET Radiopharmacy—Nuclear Medicine, Azienda Ospedaliero, Universitaria di Bologna, S. Orsolo-Malpighi Hospital, Via Massarenti 9, 40318 Bologna, Italy*
[b]*Medical Physics, Azienda Ospedaliero, Universitaria di Bologna, S. Orsolo-Malpighi Hospital, Via Massarenti 9, 40318 Bologna, Italy*

Received 19 July 2007; received in revised form 23 November 2007; accepted 2 December 2007

Abstract

Somatostatin receptors 1–5 are over expressed in neuroendocrine tumours (NETs). ^{68}Ga-labelled [1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid]-1-Nal3-Octreotide (DOTA NOC), a recent synthesized somatostatin analogue, shows high affinity for those receptors. Herein, modifications of a commercial module for the labelling of DOTA NOC with ^{68}Ga, as well as the assessment of time course of the radiochemical purity variation are described. The evaluation of radiochemical stability was done by two different chromatographic methods: reversed-phase radio HPLC and fast TLC analysis. Labelled compound has been found radiochemically stable within 3 h from the end of labelling (EOL) and radiochemical purity was always higher than 99%. After 73 labelling sessions the system showed great reproducibility and high radiochemical yield.

Keywords: PET radiopharmaceuticals; ^{68}Ga-labelled DOTA NOC; NET; Somatostatin receptors; Radiochemical stability; Quality control

1. Introduction

Somatostatin is a peptidic hormone which is present in two biologically active molecular forms: Somatostatin-14 and the N-terminally extended derivative, Somatostatin-28. Its effects include inhibition of several hormonal secretions, modulation of neurotransmission and cell proliferation. Human somatostatin receptors (Hsstr) are all metabotropic receptors and five different subtypes have been characterized and cloned (Hsstr1→5) so far (Breeman et al., 2001).

All receptors are known to be over expressed in different tumours, especially in neuroendocrine tumours (NETs) and their metastases, with Hsstr2 being the most important one (Reubi et al., 2001). Radiolabelled somatostatin analogues tracers allows the in vivo visualization of those tumours by PET or SPET scans (Behr et al., 1999; Prasad et al., 2007).

The most recent somatostatin analogue is [1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid]-1-Nal3-Octreotide (DOTA NOC) (Wild et al., 2005).

This molecule results from the modification of the octapeptide Octreotide (*D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-ol*) by substitution of third Phe moiety with *Naphthyl-L-Ala*, as shown in Fig. 1.

Such analogue, conjugated with ^{68}Ga is a potential PET tracer showing the highest affinity for somatostatin receptors Hsstr2, Hsstr3 and Hsstr5 (Wild et al., 2003).

^{68}Ga is a short-life positron emitter (half-life 67.6 min, positron energy 2.92 MeV) currently produced by a commercial ^{68}Ge/^{68}Ga generator (Ehrhardt et al., 1978).

Aim of this study was the radiolabelling of the Octreotide analogue DOTA NOC with ^{68}Ga as well as the evaluation of the radiochemical stability of labelled complex. Simple modifications of a commercial module for ^{68}Ga DOTA NOC labelling were also introduced in order to decrease the operator dose and labelling time course.

*Corresponding author. Tel.: +39 51 6363240; fax: +39 51 6363954.
E-mail address: stefano.boschi@aosp.bo.it (S. Boschi).

0969-8043/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.apradiso.2007.12.001

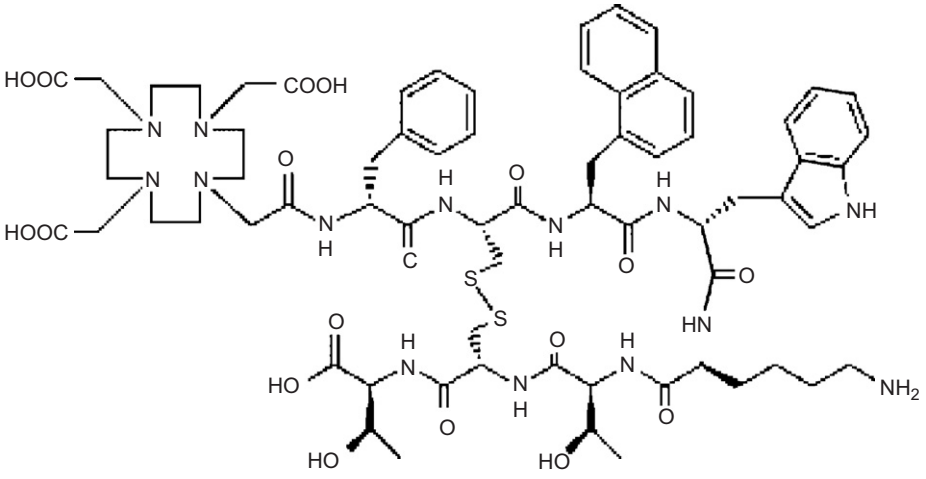


Fig. 1. DOTA NOC molecular structure.

2. Material and methods

2.1. Reagents and solvents

All solvents were of pharmaceutical grade. Trisodium citrate (pro-analysis), 0.1 M HCl (Titrisol), trifluoroacetic acid (TFA) were HPLC grade, obtained from Merck. DOTA NOC · HCl salt was purchased from Advanced Biochemical Compounds (ABX). Cation exchanger resin AG 50W-X8 (400 mesh) was obtained from Biorad and C18 Strata-X columns (30 mg) were purchased from Phenomenex. Flash TLC TecControl (Black strips) were purchased from Biodex Medical System Inc., whereas ITLC-SG were from PALL Life Science. The sterile filters MILLEX GV 0.22 μm were obtained from Millipore. HPLC grade water (resistivity = 18.2 MΩ cm) was produced by Simplicity 185 Millipore system.

2.2. Labelling protocol and system description

$^{68}Ge/^{68}Ga$ generator is produced by Cyclotron Co., Obninsk, and distributed by Eckert & Ziegler F-CON Pharmaitalia ($t_{1/2}$ of ^{68}Ge and ^{68}Ga = 270.8 days and 68 min), respectively. Labelling module was purchased from Eckert & Ziegler F-CON Pharmaitalia. Labelling protocol was developed and provided by Eckert & Ziegler with the module.

The basic configuration of commercial labelling module requires the connection of the C18 purification cartridge to a 20 mL syringe following by manual aspiration of reaction mixture containing ^{68}Ga DOTA NOC from the reaction vessel onto the cartridge. Subsequently, the C18 cartridge was mounted on a 10 mL syringe and washed with 3 mL of injectable water and 7 mL of air, to eliminate free Ga^{3+} traces. Finally, the purified ^{68}Ga DOTA NOC was eluted, by 0.8 mL of absolute ethanol, and sterilized through a sterile 0.22 μm filter.

All these steps were normally performed by holding the C18 cartridge by hands.

In order to reduce the dose, the reactor vessel was connected to the C18 cartridge and the cartridge to a 10 mL trap T1. This trap was connected to a 60 mL syringe P1 acting as a remote manual pump which allowed the reaction mixture to be loaded on the C18 cartridge by simply aspirating with the syringe (Fig. 2).

All tubings were made by Teflon, except the line to reaction vessel which was made by PEEK, and valves that were in PE. Since it is mandatory to avoid iron contamination, syringes without steel needles were used throughout the protocol.

Before starting the labelling procedure, all tubes and valves were washed with 10 mL of absolute ethanol, 30 mL of air, 10 mL of sterile water and 30 mL of air to clean the entire system and avoid bacteric and pyrogenic contaminations.

The labelling protocol, developed and supplied from Eckert & Ziegler F-CON Pharmaitalia, was modified as following. The generator was eluted with 7 mL of 0.1 N HCl and the eluate was loaded on a cationic exchange column KX. The column was then washed with 1 mL of a washing solution (0.150 M HCl in 80%/20% acetone/water solution).

After switching the valve V1 (Fig. 2) to connect the column to the reaction vessel RV, the ^{68}Ga fraction was eluted with 0.4 mL of elution-solution (0.050 M HCl in 97.56%/2.44% acetone/water solution) in the reactor vessel, set at 124 °C, containing 5 mL of the reaction mixture (50 μg of DOTA NOC · HCl in 5 mL of HPLC grade water). This setting allows the reaction mixture to reach 100 °C throughout the reaction time. Incubation time was 10 min.

Reaction mixture, containing ^{68}Ga DOTA NOC with traces of residual-free ^{68}Ga and unlabelled DOTA NOC, was then aspirated onto the C18 purification cartridge and collected in the T1 trap vial (Alltech). This step was performed using a home-made manual pump system described above and showed in Fig. 2. Column was washed with 3 mL of injectable water and dried with 7 mL of air. Valve V2 was turned to the collecting vial CV, then ^{68}Ga

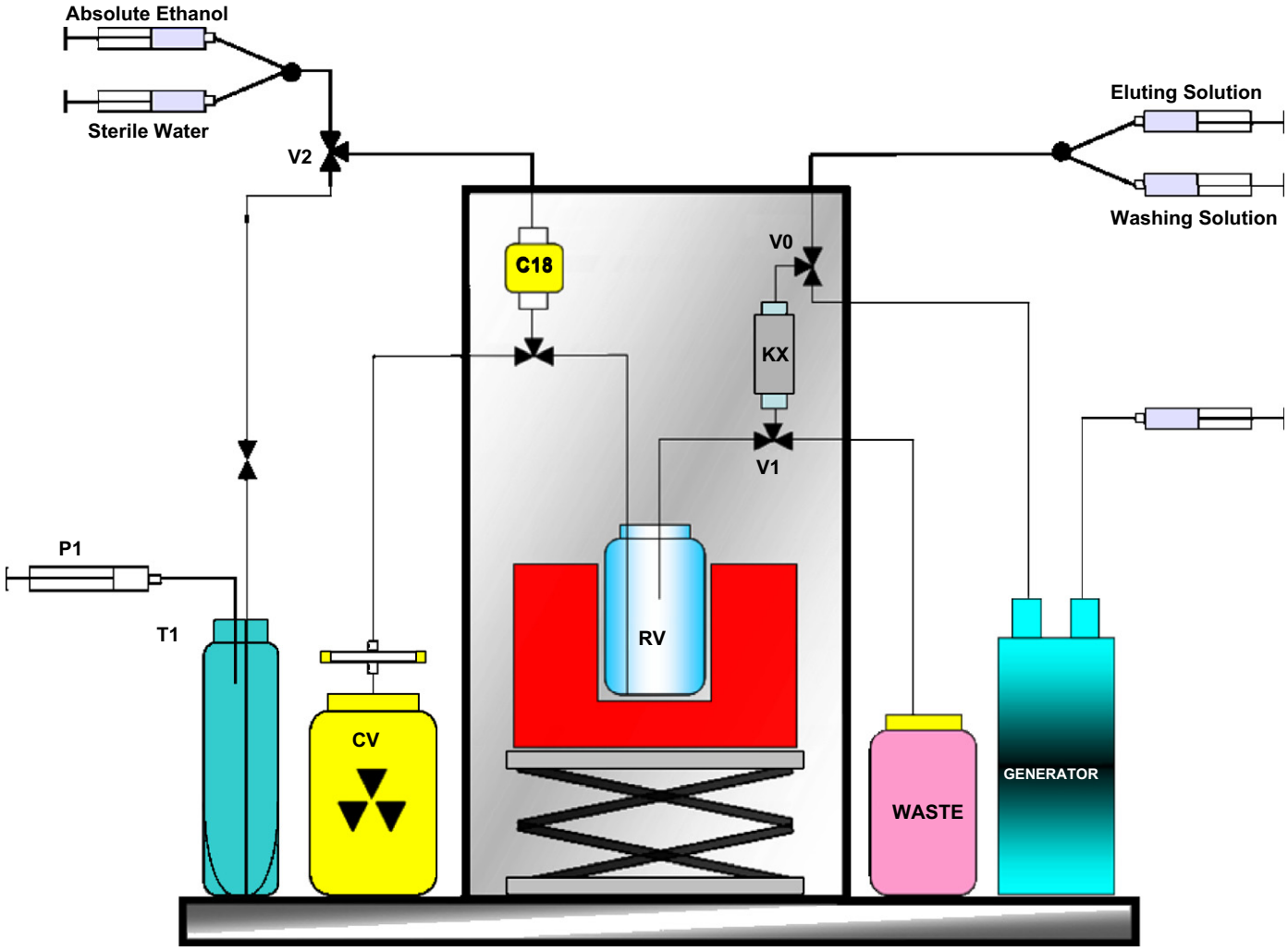


Fig. 2. Graphical rendering of the labelling module configuration with our improvements.

DOTA NOC was eluted with 0.5 mL of absolute ethanol. Final washing of the column with 8 mL of saline ensured quantitative recovery of labelled peptide from C18, lines and filter membrane. Sterilization was achieved by filtration through 0.22 μm membrane.

2.3. Quality control of $^{68}Ge/^{68}Ga$ generator

A TiO_2 column generator type has been used. The generator was specified to have an elution yield >60% and a radionuclidic purity >99%. Measurement of eluted activity was done by means of an activity calibrator (Capintec CRC15 PET). Eluting the generator the day before the labelling session was mandatory to eliminate accumulated ^{68}Zn in the column as well as to measure the eluted activity, which will be used for radiochemical yield determination.

High-resolution gamma ray spectrometry has been performed, on the whole effluents, in order to determine the radionuclidic purity.

The radiochemical purity of the eluate has been checked by means of radio HPLC in the same conditions used for ^{68}Ga DOTA NOC quality control analysis, the results were expressed as percentage of Ga^{3+} vs $[GaCl]_4^-$.

Table 1
Radiochemical purity values at different time EOL

HPLC analysis		ITLC-SG analysis		Flash-TLC analysis	
Time of injection	R.P.	Time of analysis	R.P.	Time of analysis	R.P.
EOL	>99	EOL	>99	EOL	>99
1 h EOL	>99	1 h EOL	>99	1 h EOL	>99
2 h EOL	>99	2 h EOL	>99	2 h EOL	>99
3 h EOL	>99	3 h EOL	>99	3 h EOL	>99

Different chromatographic approaches shows purity always higher than 99%.

2.4. Quality control of ^{68}Ga-DOTA·NOC

Radiochemical yield has been calculated as percentage of the activity eluted from the generator the day before labelling, and corrected for the decay.

Radiochemical purity analysis was assessed with two different chromatographic approaches, radio HPLC and TLC by using two different supports: ITLC-SG and commercial (Flash-TLC Black). HPLC technique was used also to validate ITLC approach.

2.4.1. Radio HPLC method

Radiochemical and chemical purity of ^{68}Ga DOTA NOC were determined by HPLC (Agilent, Mod.1100) with Nucleosil C18 column (4×250 mm, Knauer) using CH_3CN/H_2O/0.1% TFA linear gradient (from 5% to 90%

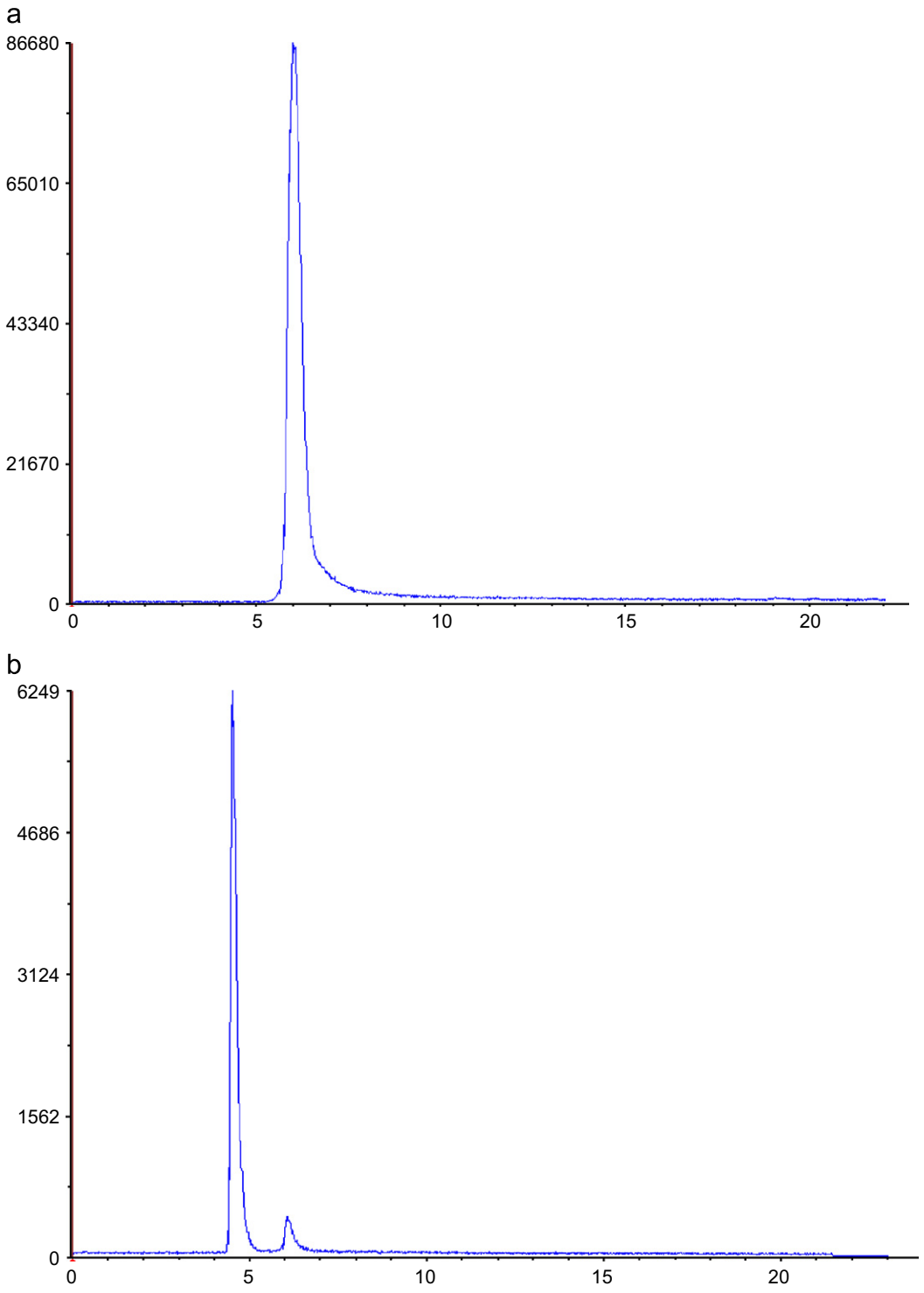


Fig. 3. (a) Radio-HPLC of generator's eluate. $^{68}Ga^{3+}$ retention time is 6.2 min and (b) Radio-HPLC of eluate brought at pH <1. Signal at 4.6 min corresponds to $[^{68}GaCl_4]^-$ complex.

of CH_3CN in 15 min) as a mobile phase at a 1 ml min^{-1} flow rate. ^{68}Ga DOTA NOC (retention time 10.4 min) was monitored by radiometric BGO detector (500TR, Packard) and UV detector (254 nm).

2.4.2. TLC methods

The radiochemical purity of ^{68}Ga DOTA NOC was determined by Flash-TLC TecControl strips and ITLC-SG, using 0.1 M trisodium citrate/0.2 M HCl as a mobile phase. Strips were analysed by autoradiography (IstantImager, Packard Instrument).

2.4.3. Others

pH of sterile solution was measured by a standard pH meter.

Endotoxins contents was evaluated by LAL test, using a gel-clotting method.

3. Results

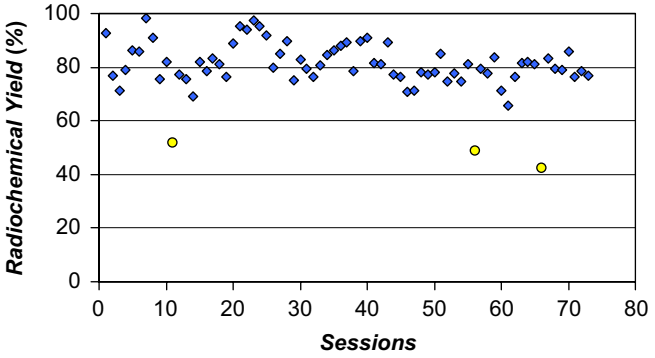
All HPLC analysis were done by injecting 10 μL, whereas both ITLC-SG and Flash-TLC TecControl were spiked with 5 μL drop, using glass 25 μL syringe (Hamilton). In order to check the stability of the radiochemical purity, 20 production batches were analysed ($n = 20$), at the end of labelling (EOL) (usually 22 min) and after 1, 2 and 3 h from the EOL.

HPLC runs did not show any changes in radiochemical purity during the time course considered for the analysis (Table 1): value was always higher than 99%. Retention time of ^{68}Ga DOTA NOC was 10.4 min, while free ^{68}Ga traces eluted at 4.8 min.

ITLC-SG and Flash-TLC were always done simultaneously and in the same chromatographic condition. As radio HPLC analysis, radiochemical purity did not show any significant variation during the time course studied, and was always higher than 99% (Table 1).

The solution flushed back into the reactor vessel after C18 purification was also analysed by radio HPLC at the EOL. As shown in Fig. 4, traces of ^{68}Ga DOTA NOC were found even in presence of a several fold higher free ^{68}Ga peak. These data suggest that some labelled peptide is not retained by the column and that cartridges with higher capacity should be useful for quantitative recovery.

The pH value of injectable product, measured by standard pH meter, was always between 5 and 5.5.



N° of Labelling	N° of scans	Average yield (%)	Range of yield (%)
73	125	81.6 ± 6.5	42 – 98

Fig. 5. Radiochemical yield distribution of 73 labelling sessions. The three outliers were occurred as complication during the C18 cartridge purification step.

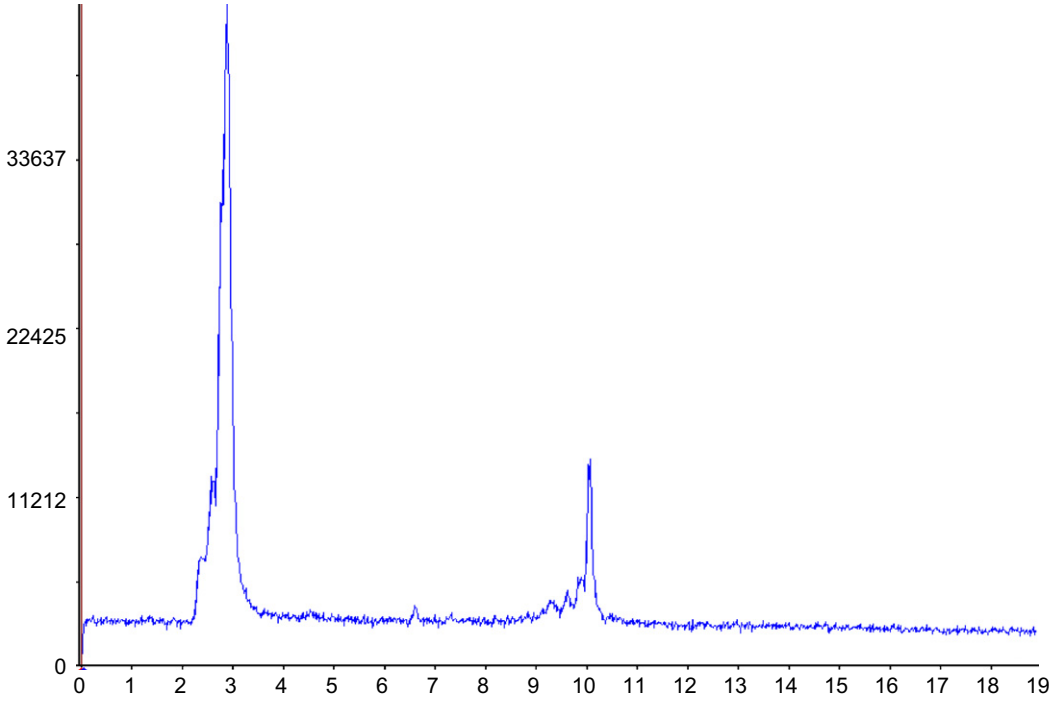


Fig. 4. Radio-HPLC reaction mix in reaction vessel after C18 cartridge purification. Only traces of ^{68}Ga DOTA NOC (rt = 10.4 min) are revealed.

Pyrogen tests were always negative, as for dilution factor 1:50.

Quality controls of eluates from the generator showed radionuclidic purity never below 99.99% and the radiochemical purity was found higher than 99.5%, in terms of trivalent cationic form of Gallium. No $[GaCl_4]^-$ complex was found in the eluate. To confirm the data, 1 mL of generator's eluate was brought to $pH < 1$ with concentrated HCl, value at which $[GaCl_4]^-$ form is prevalent. The results are shown in Fig. 3a.

The labelling procedure of ^{68}Ga DOTA NOC was also used in clinical practice. After performing 73 syntheses (corresponding to 125 patients), the radiochemical yield was found equal to: $81.6\% \pm 6.5$ (mean $\pm$ standard deviation). The distribution of radiochemical yield of all sessions is shown in Fig. 5.

4. Discussion

The ^{68}Ge/^{68}Ga generator system, with the simple labelling procedure allowed by the described system, represents an attractive solution for nuclear medicine centers without installed cyclotron. With this system, indeed, it can be feasible to produce ^{68}Ga-labelled PET tracers for routine practice, with lower costs compared with the conventional techniques (i.e. ^{111}In-Octreotide).

The proposed method for the labelling of ^{68}Ga DOTA NOC was demonstrated to be reliable and reproducible, with high radiochemical yield. Outliers results can be always referred to a losses of labelled product because of leaking fittings in the C18 purification step.

Radio HPLC and the two TLC methods had shown comparable results: radiochemical purity was always higher than 99% and remained stable during the 3 h following the EOL underlying that the ^{68}Ga DOTA NOC is a stable compound and no radiolytic phenomena did occur in the observed time-frame. This represents a reasonable time-frame for clinical use of such a short half-life radionuclide and makes this tracer suitable for multiple patients scans in the same labelling session.

This is even more evident by using the generator with great activity yield (such as 50 mCi or more) already available on the market.

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 routinely quality controls of this radiopharmaceutical.

All the batches showed acceptable pH value and were found to be sterile and negative to pyrogen test.

Concerning the operator radioprotection, we have found a significant dose reduction to operator's hands with respect to the original configuration. Measured dose to the body was reduced from 15 to 6 μSv for each complete labelling process (doses were measured by standard DOSICARD, Canberra Eurisys).

Quality controls for the considered generator eluates has shown how that system produce ^{68}Ga with radionuclidic and radiochemical purity in line with standards for radiopharmaceutical practices.

We can conclude that this system provide a reproducible, high throughput and reliable tool for routine clinical practices

References

Breeman, W.A.P., de Jong, M., Kwekkeboom, D.J., 2001. Somatostatin receptor-mediated imaging and therapy: basic science, current knowledge, limitations and future perspectives. Eur. J. Nucl. Med. 28, 1421–1429.

Behr, T.M., Behe, M., Becker, W., 1999. Diagnostic applications of radiolabeled peptides in nuclear endocrinology. Q. J. Nucl. Med. 43, 268–280.

Ehrhardt, G.J., Welch, M.J., 1978. A new germanium-63/gallium-68 generator. J. Nucl. Med. 19 (8), 925–929.

Prasad, V., Fetscher, S., Baum, R.P., 2007. Changing role of somatostatin receptor targeted drugs in NET: nuclear medicine's view. J. Pharm. Pharm. Sci., 321s–337s.

Wild, D., Macke, H.R., Waser, B., Reubi, J.C., Ginj, M., Rasch, H., Muller-Brand, J., Hofmann, M., 2005. ^{68}Ga-DOTANOC: a first compound for PET imaging with high affinity for somatostatin receptor subtypes 2 and 5. Eur. J. Nucl. Med. Mol. Imag. 32, 724.

Wild, D., Schmitt, J.S., Ginj, M., Macke, H.R., Bernard, B.F., Krenning, E., 2003. DOTA NOC, a high-affinity ligand of somatostatin receptor subtypes 2, 3 and 5 for labelling with various radiometals. Eur. J. Nucl. Med. Mol. Imag. 30, 1338–1347.

Reubi, J.C., Waser, B., Schaer, J.C., Laissue, J.A., 2001. Somatostatin receptor sst1–sst5 expression in normal and neoplastic human tissues using receptor autoradiography with subtype-selective ligands. Eur. J. Nucl. Med. 28, 836–846.