Asia Oceania Journal of Nuclear Medicine & Biology
http://aojnmb.mums.ac.ir
AOJNMB

Lutetium-177 DOTATATE Production with an Automated Radiopharmaceutical Synthesis System

Alireza Aslani[1,2]*, Graeme M Snowdon[1], Dale L Bailey[1,3], Geoffrey P Schembri[1,2], Elizabeth A Bailey[1], Nick Pavlakis[2,4], Paul J Roach[1,2]

[1] Department of Nuclear Medicine, Royal North Shore Hospital, Sydney, Australia
[2] Sydney Medical School, University of Sydney, Sydney, Australia
[3] Faculty of Health Sciences, University of Sydney, Sydney, Australia
[4] Department of Medical Oncology, Royal North Shore Hospital, Sydney, Australia

ARTICLE INFO

Article type:
Technical note

Article history:
Received: 17 Feb 2015
Revised: 27 Apr 2015
Accepted: 30 Apr 2015

Keywords:
Automated synthesis
Lutetium-DOTATATE,
Neuroendocrine tumours
Peptide Receptor Radionuclide Therapy

ABSTRACT

Objective(s): Peptide Receptor Radionuclide Therapy (PRRT) with yttrium-90 (^{90}Y) and lutetium-177 (^{177}Lu)-labelled SST analogues are now therapy option for patients who have failed to respond to conventional medical therapy. In-house production with automated PRRT synthesis systems have clear advantages over manual methods resulting in increasing use in hospital-based radiopharmacies. We report on our one year experience with an automated radiopharmaceutical synthesis system.
Methods: All syntheses were carried out using the Eckert & Ziegler Eurotope's Modular-Lab Pharm Tracer® automated synthesis system. All materials and methods used were followed as instructed by the manufacturer of the system (Eckert & Ziegler Eurotope, Berlin, Germany). Sterile, GMP-certified, no-carrier added (NCA) ^{177}Lu was used with GMP-certified peptide. An audit trail was also produced and saved by the system. The quality of the final product was assessed after each synthesis by ITLC-SG and HPLC methods.
Results: A total of 17 [^{177}Lu]-DOTATATE syntheses were performed between August 2013 and December 2014. The amount of radioactive [^{177}Lu]-DOTATATE produced by each synthesis varied between 10-40 GBq and was dependant on the number of patients being treated on a given day. Thirteen individuals received a total of 37 individual treatment administrations in this period. There were no issues and failures with the system or the synthesis cassettes. The average radiochemical purity as determined by ITLC was above 99% (99.8 ± 0.05%) and the average radiochemical purity as determined by HPLC technique was above 97% (97.3 ± 1.5%) for this period.
Conclusions: The automated synthesis of [^{177}Lu]-DOTATATE using Eckert & Ziegler Eurotope's Modular-Lab Pharm Tracer® system is a robust, convenient and high yield approach to the radiolabelling of DOTATATE peptide benefiting from the use of NCA ^{177}Lu and almost negligible radiation exposure of the operators.

▶ Please cite this paper as:
Aslani A, Snowdon GM, Bailey DL, Schembri GP, Bailey EA, Pavlakis N, Roach PJ. Lutetium-177 DOTATATE Production with an Automated Radiopharmaceutical Synthesis System. Asia Oceania J Nucl Med Biol. 2015; 3(2): 107-115.

Introduction

Neuroendocrine tumours (NETs) comprise a wide spectrum of tumours arising from neural crest cells throughout the body. While once considered rare, recent epidemiologic data has

* *Corresponding author:* Alireza Aslani, Department of Nuclear Medicine, Royal North Shore Hospital, St Leonards, Sydney, Australia.Tel: +61 2 99264440; Fax: +61 2 99264099; E-mail: alireza.aslani@health.nsw.gov.au

shown increasing incidence with little change in mortality, suggesting that there has been significant under-diagnosis in the past (1). These tumours are often slow growing and may go undiagnosed for long periods of time leading to patients presenting with advanced disease. Symptoms are frequently vague leading to delays in diagnosis. The appropriate management of these tumours is best handled through a multidisciplinary approach as their diagnosis and treatment crosses a range of specialities.

Neuroendocrine gastrointestinal tumours are the most common. Terminology has been varied over the years and these are often broken down into carcinoid (mid-gut) tumours and endocrine pancreatic neoplasms. While these two groups have different behaviour, they are often encompassed in the single term - gastroenteropancreatic neuroendocrine tumours (GEP-NETs). Many of these tumours (the so called carcinoid tumours) produce endocrine syndromes due to the overproduction of a range of hormones.

The fact that NETs overexpress subtypes of somatostatin (SST) receptors (2) allows for diagnosis and treatment with SST analogues. While all five SST receptor subtypes are overexpressed to some degree (3), SST_2 is the most widely overexpressed. Peptide Receptor Radionuclide Therapy (PRRT) has gained substantial interest in the last few years (4). Radiolabelled somatostatin analogues are now considered for patients who have failed to respond to conventional medical therapy (5, 6). The two most commonly used radioisotopes are yttrium-90 (^{90}Y) and lutetium-177 (^{177}Lu). These radioisotopes differ in terms of their emitted radiations, particle energy, physical half-life and tissue penetration (7, 8). The radiopharmaceuticals being used for this therapy are DOTATATE, DOTATOC and DOTANOC, all of which are labelled via the DOTA chelator to either ^{90}Y or ^{177}Lu (7). These radiopharmaceuticals have a range of SST affinities though all have good affinity for SST_2. Although the synthesis and purification of these radiopharmaceuticals can successfully be carried out manually in the laboratory, the use of automated synthesis systems is gradually increasing (9). These automated systems have clear advantages over manual methods which have resulted in their increasing installation and use in hospital-based radiopharmacies. At our institution we use no-carrier added (NCA) ^{177}Lu labelled with a locally synthesised peptide for the PRRT of patients with well-differentiated NETs. The [^{177}Lu]-DOTATATE (referred to as "NCA-LuTATE") is synthesised in-house using an automated synthesis system. Here we report on our one year, 17 syntheses experience with this formulation.

Methods

There are a number of reports in the literature using and detailing various automated radiochemistry systems used for synthesising radio-pharmaceuticals (RP) labelled with gallium-68 (^{68}Ga), ^{177}Lu and other radioisotopes (10-12). However, the number of reports in the literature using automated synthesis systems for [^{177}Lu]-DOTATATE is scarce except for de Decker and Turner (13) who recently reported their experience of automated synthesis systems for [^{177}Lu]-DOTATATE synthesis. Automated RP synthesis systems use generic synthesis procedures with minor modifications tailored to the specific needs of the institution and can also include chemical analysis and detection systems such as High Pressure Liquid Chromatography (HPLC) units. As a result, over time, these setups evolve and are modified to improve productivity and can become unique to the institution. The system reported here was the Eckert & Ziegler Eurotope's Modular-Lab Pharm Tracer® automated synthesis system.

All materials and methods used were followed as initially instructed or later modified by the manufacturer of the system (Eckert & Ziegler Eurotope, Berlin, Germany - subsequently referred to as the vendor or manufacturer) from whom the automated RP synthesis system and synthesis cassettes were obtained.

Reagents

As recommended by the manufacturer, all reagents were high purity pharmaceutical grade, unless stated otherwise. The exact grade as well as the source of the reagents used was also their recommendation.

Water puriss p.a (FLUKA Trace SELECT® 95305) used in the preparation of the majority of reagents was obtained from Sigma-Aldrich, Australia. Sodium chloride 0.9% for injection in 50 mL glass bottles (INJ089) were obtained from Phebra (Lane Cove, NSW, Australia). The L-ascorbic acid puriss p.a (FLUKA 33034-100 G), sodium hydroxide monohydrate (Trace SELECT® 01968-25 GF; ≥99.9995% pure), and 200 proof ethanol (HPLC / spectrophotometric grade, 459828-1L) were also obtained from Sigma-Aldrich, Australia.

^{177}Lu was produced by a different method to that commonly reported previously. Two distinct methods for reactor production of ^{177}Lu can be

used: the more commonly used approach is to directly irradiate high purity ^{176}Lu via the reaction ^{176}Lu(n,γ)^{177}Lu, while the alternative is to irradiate ^{176}Yb as in ^{176}Yb(n,γ)^{177}Yb which decays with a 1.9 hour half-life to ^{177}Lu. The latter method has the advantages that (a) there is no carrier lutetium present, and so is referred to as "no-carrier added" lutetium (NCA [^{177}Lu]) , and (b) there is no ^{177m}Lu produced, which has a physical half-life of 160 days and therefore presents a significant radioactive waste storage and disposal problem. A further advantage of the NCA [^{177}Lu] is that it requires less peptide to label the necessary active amount of [^{177}Lu]-DOTATATE for the therapy. This is the production methodology for the ^{177}Lu utilised at our institution.

The DOTATATE, or DOTA-(Tyr^3)-octreotate (where DOTA = 1,4,7,10-tetraazacyclododecane–1,4,7, 10-tetraacetic acid) was obtained from Auspep (Melbourne, Australia). The peptide is provided as a 1 mg powder which is subsequently dissolved in 1 mL of water and used in 200 µL aliquots. The DOTATATE peptide is certified as Good Manufacturing Practice (GMP) grade.

The preparation of the required [^{177}Lu]-DOTATATE reagents and chemicals are shown in Table 1. This table is used as a quick instruction guide for the preparation of chemicals at our centre.

The ^{177}Lu (NCA $^{177}LuCl_3$) was obtained from ITG (Isotope Technologies Garching GmbH, Germany) through a partnership with the Australian Nuclear Science and Technology Organisation (ANSTO), Sydney, Australia. The amount of radioactive ^{177}Lu ordered for each synthesis was based on the number of patients to be treated per synthesis and an estimate of 80% synthesis yield. The prescribed dose to be injected was a notional 8 GBq per patient (14).

Synthesis Cassettes and Automation

The synthesis of [^{177}Lu]-DOTATATE can be separated into three parts. These are:

a) the modules, arranged according to the specific RP being synthesised;
b) the synthesis cassettes on which the appropriate chemicals, reagents, filters, columns, etc are attached; and
c) the computer system that executes the software which, in turn, operates the valves and syringes on the cassettes producing the required RP.

Since the system is computer-controlled, an audit trail is always maintained which is a requirement for GMP production certification. There is a specific synthesis cassette for the production of [^{177}Lu]-DOTATATE. The same system is also currently being used for the production of other PET RP, such as [^{68}Ga]-DOTATATE and [^{68}Ga]-PSMA (Prostate-Specific Membrane Antigen) using separate synthesis cassettes as well as another for a simple elution of the ^{68}Ge/^{68}Ga generator. All production cassettes are obtained from the vendor. These cassettes are sterile and for single-use only.

As with the cassettes, there is a specific computer template program for each RP synthesis process. These templates are also provided by the vendor. These are subdivided into programs for the cassette pressure testing, terminal sterilisation filter testing, running the HPLC analysis and, in the case of [^{68}Ga]-DOTATATE and [^{68}Ga]-PSMA, also for eluting the ^{68}Ge/^{68}Ga generator. The software also allows programming and modifications to the templates via a graphical user interface (GUI).

Lutetium-177 (^{177}Lu)

Lutetium-177 ($t_{½}$ = 6.7 days) emits both β$^-$ and γ radiation during its decay to stable ^{177}Hf. The principal β$^-$ emission has an average energy of 0.149 MeV with 78.6% abundance. There are a number of small abundance γ photons emitted, with those of interest for imaging using the gamma camera being 0.208 MeV (11% abundance) and 0.113 MeV (6% abundance). The fact that few γ photons are emitted allows many ^{177}Lu therapies to be performed on an outpatient basis from a radiation safety perspective in many jurisdictions despite the patient being administered up to 8-10 GBq of ^{177}Lu, with exposure from contact with treated patients being comparable to that seen with a ^{99m}Tc bone scan. The ^{177}Lu we have used for all syntheses was sterile, GMP-certified, no-carrier added ^{177}Lu in the form of lutetium chloride ($^{177}LuCl_3$) and is supplied in a volume of 0.5 mL (0.04 M HCl solution). The radioisotope generally arrives at our centre one day prior to the actual day of synthesis. The radioactivity was calibrated for the day and time the synthesis was due to be carried out. The radioactivity ordered was requested to be approximately 10 GBq per patient which allowed for an approximate 80% product yield providing a final 8 GBq dose per patient. To date, up to four patient doses have been produced in a single synthesis, i.e., an approximate amount of 40 GBq of NCA ^{177}Lu at start of synthesis.

Automated Radiolabelling Process

Once the sterile synthesis cassette is removed from its packaging and all connections tightened, it is attached to the Modular Lab's synthesis cassette

Table 1. Checklist of chemicals used for [^{177}Lu]-DOTATATE synthesis

Name of Solution	Preparation	Product Code
Ascorbic Acid Buffer stock solution (0.57 M, pH 4.5)	Prepare 2 solutions Solution 1: Dissolve 2.2 g ascorbic acid in 22 mL water (puriss p.a.) Solution 2: Dissolve 0.9 g sodium hydroxide in 2.1 mL water (puriss p.a.) Adjust pH to 4.5± 0.1: Add 1 mL of solution 2 to solution 1. Use 0.5 mL sample to check pH. Discard sample after measurement. Add more solution 2 in small steps and measure pH of samples (0.5 mL). Discard samples after measurement. Stop adjusting at pH to 4.5± 0.1. Inject buffer samples through sterile filter (0.2 µm) aliquots into dry sterile vials. Radiolabelling volume: 2-5 times high volume Ascorbic buffer with respect to radioactivity volume. Store at -20 °C not longer than 1 month	Ascorbic Acid, Sigma Aldrich 33034-100 G Sodium hydroxide, Sigma Aldrich 01968-25 G-F Water Puriss. p.a., FLUKA 95305
Ethanol solution	9.5 mL ethanol 10.5 mL H_2O	Ethanol 200 proof HPLC/Spectrophotometric grade Sigma Aldrich 459828-1L Water Puriss. p.a., FLUKA 95305
NaCl (saline)	50 mL sterile saline	Sodium Chloride 0.9% Injection 900 mg in 50 mL Phebra INJ072
DOTATATE	Stock DOTATATE XX mg peptide in XX mL H_2O; Optimum conditions for ^{177}Lu labelling: 1 µg DOTA peptide per 40 MBq of ^{177}Lu. e.g., 8 GBq dose: 200 µg DOTA peptide. Store in freezer Dispense 200 mL into eppendorf vials. Store in freezer	DOTA-(TYR3) Octreotate acetate salt 2500437 1 mg CAS-No 177943-89-4 Water Puriss. p.a., FLUKA 95305
[^{177}Lu]-DOTATATE ITLC QC	0.1 M Na Citrate pH 5.0 100 mL	

module. The radiolabelling process is performed in three steps. The first step tests the cassette for any leaks. This step, also known as the Cassette Pressure Test, applies a pressure of 200 kPa to various sections of the cassette. This is software controlled. This test is performed by attaching the cassette to a high purity nitrogen gas supply with its pressure regulator adjusted to deliver 200 kPa of pressure. A visual progress graph is also displayed (Figure 1). If there is no loss of pressure to below 100 kPa, the test is considered to have passed and the cassette is suitable for use. However, if the test fails, the cassette is removed from the module, all connections re-tightened and test repeated. If the test fails again, the cassette is rejected and sent back to the manufacturer and replaced. The process of pressure testing the cassette takes approximately five minutes. On completion, the cassette pressure test process log is saved on the computer for audit trail purposes. On passing the test, the necessary pre-prepared reagents are placed in the appropriate containers of the synthesis cassette and ^{177}Lu vial connected. The empty containers, the waste bottle and product vial are then attached. The product vial and waste bottle are placed in a specially designed Perspex container boxes to reduce any β^- radiation dose to the operator. The vial containing the ^{177}Lu is kept in its original lead shielded container also to reduce radiation dose to the operator. The second step of the synthesis phase is similarly driven by the Modular Lab Pharm Tracer® software. The duration of this process is approximately 45 minutes and is the main step of the entire process. The movement of all liquids is driven by a 10 mL syringe attached to the syringe-driver module. This phase involves a number of different steps, including washing of various sections with 0.9% saline. Briefly, the Waters tC18 Light reverse phase silica cartridge (also known

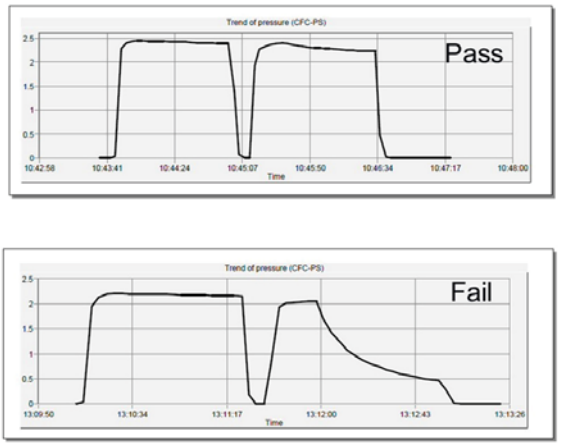
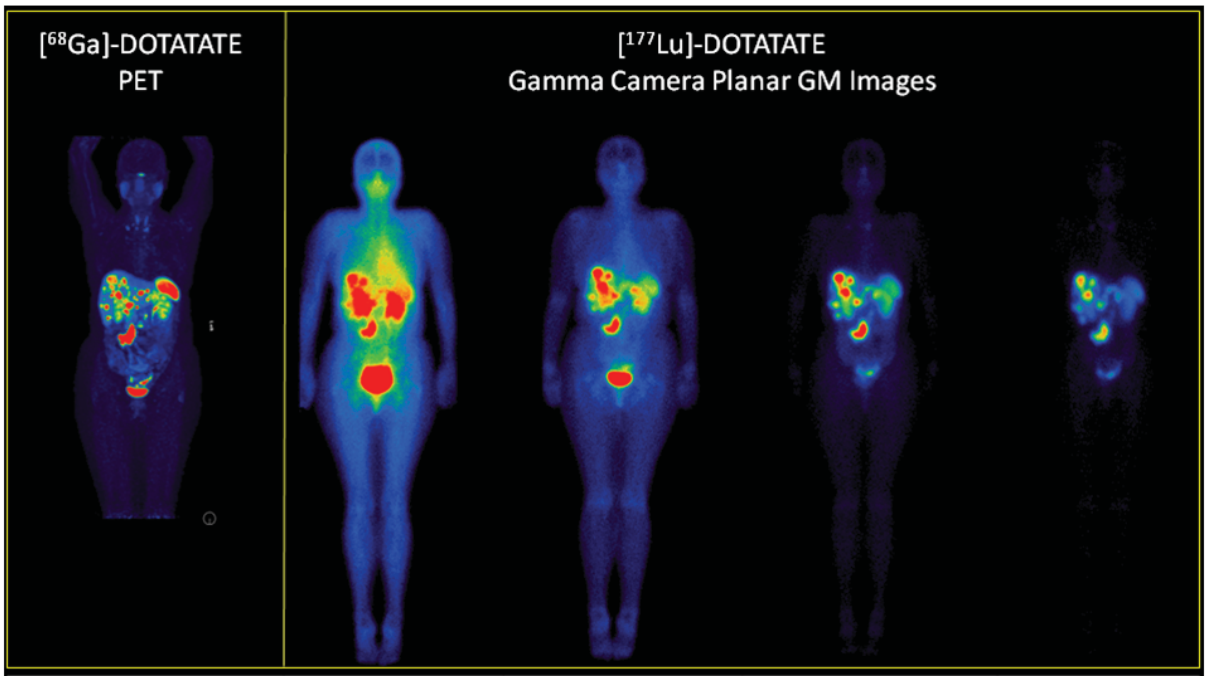


Figure 1. Pressure test trace indicating a pressure test Pass (top) and a Fail (bottom)

as C18 ion exchange or SepPak® cartridge) is initially primed (wetted) with ethanol followed by 0.9% saline. The [^{177}Lu]-LuCl$_3$ is then transferred from its vial (radioactivity vial) into the reactor which contains the DOTATATE peptide. To ensure that the entire radioactivity has been removed from the delivery vial, it is then washed with the buffer solution and the remaining radioactivity is transferred to the reaction vial. Once all the lutetium is in the reaction vial together with the peptide, the mixture is heated at 80° C for 20 min. The cassette is then rinsed twice with 0.9% saline to remove any residual radioactivity from the fluid paths. The crude product from the reaction vial is then removed and kept in the syringe for five minutes to cool. Once cooled, it is then passed through the C18 cartridge to remove any unbound [^{177}Lu]-LuCl$_3$. Both the C18 cartridge as well as the reaction vial are then rinsed with 0.9% saline to recover any residual product. The product, now trapped in the C18 cartridge, is eluted by first passing an ethanol / water mixture (47.5%:52.5% ratio) and then 0.9% saline into the final product vial. To ensure sterility, the final product is passed through a 0.22 µm filter. The total volume of the final product is 18 mL. The software provides a graphical display of each step and progress of the synthesis. On completion, the synthesis process is digitally saved on the computer for audit purposes.

The third phase of the synthesis involves testing of the integrity of the 0.22 µm filter. This is known as the Filter Pressure Test and is, again, driven by the Modular Lab Pharm Tracer® software. The only operator intervention required is the removal of the 0.22 µm filter and needle from the product vial and putting it into the waste bottle prior to the test. The test is performed by subjecting the filter



Time after injection (hrs)	0.5	2.3	20.8	92.9
Radioactivity retained (MBq)	6378	2745	997	484

Figure 2. Example of representative images from a single individual showing [^{68}Ga]-DOTATATE PET scan (MIP image) (left) and after infusion of ~6.4 GBq of [^{177}Lu]-DOTATATE at four time points. The gamma camera images are geometric mean (GM) images that have been corrected for attenuation using a pre-acquired transmission scan and converted to units of radioactivity (kBq)
The distribution of disease on [^{177}Lu]-DOTATATE images is identical to that seen on the preceding diagnostic [^{68}Ga]-DOTATATE scan

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.