Investigation into various aspects of radiolabelling somatostatin peptide derivatives with ^{68}Ga eluted from a SnO_2-based $^{68}Ge/^{68}Ga$ generator

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DECLARATION

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ABSTRACT

$^{68}Ge/^{68}Ga$ generators ensure the supply of ^{68}Ga for positron emission tomography (PET), for instance for somatostatin receptor imaging with ^{68}Ga-DOTA-labelled somatostatin analogues. There are various generators available and their eluates are processed differently for radiolabelling of peptides.

The objectives of this study were to investigate various aspects of the elution characteristics of the generator, to optimize labelling conditions using different eluate processing techniques such as fractionation and cation exchange chromatography and to develop user-friendly kit formulations.

This study was approved by the Stellenbosch University Health Research Ethics Committee and permission was granted for the experimental work to be conducted at iThemba LABS. Elution efficiencies were determined using different HCl concentrations (0.2 M – 1.0 M). Metal analysis and ^{68}Ge breakthrough determination were performed on eluates. Radiolabelling parameters were optimized, using fractionated eluates and different DOTA-peptide masses (15 to 50 µg) at pH 3.5 – 4.0 in sodium acetate buffer. Different heating times and heating methods and the influence of various periods of non-elution of the generator on radiolabelling results were investigated. Cationic resins were investigated for eluate processing. Radiolabelling parameters, using cationic resin-processed eluates, were optimized. Labelling was conducted at various pH values, using different quantities of buffer. DOTA-peptide kits for both fractionated and resin-processed eluates were developed and tested for sterility, endotoxin content and stability. Radiochemical yields, radiolabelling efficiency and radiochemical purity of ^{68}Ga-DOTA-peptides were determined.

The elution efficiency of the generator increased with an increase in the concentration of HCl eluent. The ^{68}Ge breakthrough increased dramatically at 0.8 M HCl. Most metal contaminants were lowest when eluting with 0.2 M HCl and the Zn content increased with the increase in HCl concentration. The eluent of choice for the SnO_2-based generator was confirmed to be 0.6 M HCl.

For radiolabelling, 35 µg DOTA-peptide (9.2 – 9.4 µM) was the most favourable. Extended heating times and heating method did not significantly impact on the radiolabelling. The radiolabelling efficiencies were consistently above 90 % even after 3 weeks of non-elution of the generator, but radiochemical yields dropped after 7 days. DOTA-peptide kits for fractionated eluates were successfully developed and the radiolabelling quality was found to be superior over peptide stock solutions.

A radiolabelling method using a cationic exchange resin was successfully adapted for the SnO_2 generator. ^{68}Ga was efficiently adsorbed on a Bond Elut SCX (100 mg) cartridge and desorbed by acidified solutions of NaCl. The SCX resin effectively removed about 98 % of deliberately "spiked" metals from the ^{68}Ga eluate. An optimized labelling method based on the use of SCX-purified eluates was developed, producing radiochemical yields of almost 85 % and lead to the successful formulation of DOTATATE kits. The quality was found to be suitable over a 3-month period.

In conclusion, a kit type labelling procedure, using cationic resin purified ^{68}Ga eluates, provides the most practical method to produce ^{68}Ga-labelled DOTA-peptides.

OPSOMMING

$^{68}Ge/^{68}Ga$ generators verseker beskikbaarheid van ^{68}Ga vir positron emissie tomografie (PET), bv. vir somatostatien reseptor beelding met ^{68}Ga-DOTA-gemerkte somatostatien analoë. Verskillende tipes generators is beskikbaar waarvan die eluate op verskillende maniere vir die radiomerking van peptiede geprossesseer word.

Die doelstellings van hierdie studie was om verskeie aspekte van die elueringskarakteristieke van die generator te ondersoek, om radioaktiewe merkingsparameters deur middel van verskillende eluaatverwerkingstegnieke soos fraksionering en katioonuitruil chromatografie te optimaliseer en om gebruikersvriendelike kitsstelformulerings te ontwikkel.

Goedkeuring vir hierdie studie is deur die Komitee vir Navorsing en Etiek van die Universiteit van Stellenbosch toegestaan en toestemming is ook deur iThemba LABS verleen vir die uitvoer van alle eksperimentele werk by hierdie fasiliteit. Elueerdoeltreffendheid met verskillende HCl konsentrasies (0.2 M – 1.0 M) is bepaal. Metaalanalises en bepalings van ^{68}Ge deurbraak is op eluate uitgevoer. Radiomerkingsparameters is geoptimaliseer met gefraksioneerde eluate en verskillende peptiedmassas (15 tot 50 µg) by pH 3.5 – 4.0 in ‘n natriumasetaat buffer. Verkillende verhittingstye en -metodes is ondersoek. Die invloed van verskeie tye van nie-eluering van die generator op radiomerking, is ondersoek. Katioonuitruilharse is ondersoek vir suiwering van eluate. Merking is by verskillende pH vlakke en met verskillende hoeveelhede buffer uitgevoer. DOTA-peptiedkitsstelle is vir beide gefraksioneerde en hars-gesuiwerde eluate ontwikkel en is vir steriliteit, endotoksieninhoud en stabiliteit getoets. Radiochemiese opbrengs, radiomerkingsdoeltreffendheid en radiochemiese suiwerheid van ^{68}Ga-DOTA-peptiede is bepaal.

Die elueringsdoeltreffendheid van die generator het stelselmatig toegeneem met ‘n toename in die konsentrasie van HCl elueermiddel. ^{68}Ge deurbraak het aansienlik toegeneem by 0.8 M HCl. Die meeste metaalonsuiwerhede was die laagste wanneer met 0.2 M HCl ge-elueer is en die Zn inhoud het konsekwent toegeneem met toename in HCl konsentrasie. ‘n HCl konsentrasie van 0.6 M HCl is bevestig as die voorkeur elueermiddel vir die SnO_2-gebaseerde generator.

Vyf-en-dertig µg DOTA-peptied (9.2 – 9.4 µM) is as optimaal vir die radiomerkingsproses gevind. Nòg verlengde verhittingstye, nòg die tipe verhittingsmetode het enige noemenswaardige invloed op radiomerkingsresultate gehad. Die radiomerkingsdoeltreffendheid was konsekwent hoër as 90 %, selfs na 3 weke van nie-eluering van die $^{68}Ge/^{68}Ga$ generator, maar radiochemiese opbrengste het

begin afneem na 7 dae. DOTA-peptied kitsstelformulerings vir gebruik met gefraksioneerde eluate is suksesvol ontwikkel en die radiomerkingsgehalte was beter as dié van peptied voorraadoplossings.

‘n Radiomerkingsmetode waarin eluate deur middel van ‘n katioonuitruilhars geprossesseer is, is suksesvol aangepas vir die SnO_2 generator, wat met hoër konsentrasies HCl ge-elueer word. Die ^{68}Ga is doeltreffend op ‘n Bond Elut SCX (100 mg) kolom ge-adsorbeer en daarna -elueer met aangesuurde NaCl oplossing herwin. Die SCX hars het ongeveer 98 % van metale wat doelbewus by eluate gevoeg is, doeltreffend verwyder. ‘n Ge-optimaliseerde merkingsmetode, gebaseer op die gebruik van SCX-gesuiwerde eluate, is ontwikkel wat radiochemiese opbrengste van nagenoeg 85 % tot gevolg gehad het en tot die suksesvolle formulering van gebruikersvriendelike DOTATATE kitsstelle gelei het. Die kwaliteit is gedurende ‘n 3 maande stoorperiode deurlopend geskik bevind.

Hierdie studie het getoon dat ‘n kitsstel vir merking met katioon-hars gesuiwerde ^{68}Ga eluate die mees praktiese metode vir bereiding van ^{68}Ga-gemerkte DOTA-peptiede is.

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I dedicate this dissertation to Neeran and Layla for their unconditional love and inspiration

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LIST OF ABBREVIATIONS

CT:	Computed tomography
DOTA:	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
DOTATATE:	1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid-D-Phe^{1}-Tyr^{3}-octreotate
DOTANOC:	1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid-1-Nal-3-Octreotide
DOTATOC:	1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid-D-Phe^{1}-Tyr^{3}-octreotide
FDG:	Fluorodeoxyglucose
HEPES:	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC:	High performance liquid chromatography
ICP-OES:	Inductively coupled plasma optical emission spectrometer
iTLC:	Instant thin layer chromatography
iTLC-SG:	Instant thin layer chromatography-silica gel
IU/ml:	International units per millilitre
LAL:	Limulus amoebocyte lysate
LE:	Radiolabelling or labelling efficiency
MRI:	Magnetic resonance imaging
NET:	Neuroendocrine tumour
PAN:	Nanoceria-polyacrylonitrile
PET:	Positron emission tomography
RC:	Radiochemical
SPECT:	Single photon emission computed tomography
SRS:	Somatostatin receptor scintigraphy
SSR:	Somatostatin receptor
TB:	Thioglycollate broth

LIST OF DEFINITIONS

Elution efficiency

The ^{68}Ga activity at the time of elution expressed as percentage of the ^{68}Ge activity on the column (nominal value) at the time of elution.

Radiochemical (RC) yield

The activity present in the purified DOTA-peptide (in this study: ethanol:saline, ex C18 cartridge), expressed as a percentage of the decay-corrected pre-labelling input activity.

Radiochemical (RC) purity

The radiochemical purity is the quantity of radiolabelled DOTA-peptide, expressed as a percentage of the total radioactivity in the sample.

Radiolabelling/labelling efficiency (LE)

The quantity of labelled DOTA-peptide, expressed as a percentage of the total amount of radioactivity species recorded on the HPLC radio-chromatogram.

Radiopharmaceutical kit

A preparation to be reconstituted and/or combined with radionuclides to form the final radiopharmaceutical preparation, usually just prior to its administration.

Chapter 1: Introduction and Literature Review

$^{68}Ge/^{68}Ga$ generator systems provide a reliable source of the positron-emitter ^{68}Ga (half-life of 68 min) which is used to produce radiopharmaceuticals for Positron Emission Tomography (PET) studies. These generators contain different sorbent materials (such as TiO_2, SiO_2 and nanoceria-polyacrylonitrile) onto which the ^{68}Ge is loaded. Additional investigation into aspects of the radiolabelling with the SnO_2 generator is required because of the different composition of the ^{68}Ga eluates obtained from these generators. These include the different acidity of the generator eluents, ^{68}Ge breakthrough, metal contaminants and the volume of the ^{68}Ga eluate. This study focuses largely on optimisation of radiolabelling procedures and increasing the user-friendliness of the labelling procedures with ^{68}Ga eluates from a SnO_2-based $^{68}Ge/^{68}Ga$ generator.

1.1 Clinical background

Neuroendocrine tumours (NETs) are a heterogeneous group of slow-growing neoplasms, arising from endocrine cells. The value of advanced diagnostic techniques lies in the improved management of the disease, in determining the extent and activity of the disease, selecting appropriate treatment (non-radioactive or radiolabelled somatostatin only if the lesions are somatostatin-avid), follow-up after treatment and disease management.

Somatostatin receptors 1 – 5 (SSRs 1 – 5) are over-expressed in NETs such as pituitary adenoma, pancreatic islet cell tumour, carcinoid, pheochromocytoma, paraganglioma, medullary thyroid cancer, and small cell lung carcinoma (Krenning et al., 1993; Reubi, 1997; Reubi, 2003). Somatostatin receptor scintigraphy (SRS) is used to detect NET lesions and in staging of NETs. Because of its superior imaging properties, PET is preferred to Single Photon Emission Computed Tomography (SPECT) for SRS. The diagnosis of patients with NET mainly relies on morphological imaging techniques such as computed tomography (CT), ultrasound (US) and magnetic resonance imaging (MRI) combined with functional imaging, including PET, which reveals physiological activity within the tumour. The study by Kayani, et al. (2008) showed that functional imaging with PET tracers containing positron emitters such as fluorine-18 (^{18}F) and gallium-68 (^{68}Ga) can provide a map of the spread of pathology in a non-invasive manner.

The diagnostic applications of ^{68}Ga-labelled DOTA-conjugated peptides for PET have been well documented (Hofmann et al., 2001; Henze et al., 2001; Meyer et al., 2003a, 2003b, 2004; Maecke et al., 2005; Breeman et al., 2005). The ^{68}Ga-DOTA-peptides have a wide clinical

importance, which is described in various studies with [^{68}Ga]DOTATOC (Kroiss et al., 2011; Henze et al., 2001; Jindal et al., 2010; Kowalski et al., 2003), [^{68}Ga]DOTATATE (Conry et al., 2010; Mojtahedi et al., 2014), and [^{68}Ga]DOTANOC (Ambrosini et al., 2008, 2010a, 2010b, 2012; Prasad et al., 2010; Wild et al., 2013). ^{68}Ga-labelled DOTA-peptides showed better preclinical and pharmacological performance than other radiotracers such as ^{111}In-labelled peptides (Antunes et al., 2007). Although uptake of the different ^{68}Ga-DOTA-somatostatin analogues in lesions differ due to different affinities for somatostatin receptor subtypes, the clinical significance of these differences and their impact on the interpretation of scans is still debated (Bozkurt et al., 2017).

[^{68}Ga]DOTA-labelled somatostatin analogues [^{68}Ga]DOTATOC (1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid-D-Phe^{1}-Tyr^{3}-octreotide), [^{68}Ga]-DOTATATE (1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid-D-Phe^{1}-Tyr^{3}-octreotate), and [^{68}Ga]DOTANOC (1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid-1-Nal3-Octreotide), have become a standard for SSR imaging and assessment of NETs using PET and PET/CT (Kwekkeboom et al., 2010; Henze et al., 2001; Hoffman et al., 2001; Meyer et al., 2004; Maecke et al., 2005; Breeman et al., 2005). The European Association of Nuclear Medicine has published guidelines for their use in imaging neuroendocrine neoplasms (Bozkurt et al., 2017).

1.2 DOTA-somatostatin analogues

DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) is an organic molecule consisting of a central 12-membered tetraaza ring which is an excellent chelating moiety for binding of metals, including gallium, and therefore, DOTA-linked peptides can be rapidly and efficiently labelled with ^{68}Ga at high specific activities (Breeman and Verbruggen, 2007). The three DOTA-derivatives DOTATOC, DOTANOC and DOTATATE display differences in receptor affinity and selectivity towards human somatostatin receptor-subtypes. All three have very high affinity for subtype 2, while DOTANOC also has very high affinity for subtype 5 (Antunes, et al., 2007; Reubi, et al., 2000). DOTANOC is more lipophilic than DOTATATE and DOTATOC (Wild et al. 2003, 2013).

The structural formulae of the three DOTA-peptides (DOTATOC, DOTANOC and DOTATATE) are illustrated below.

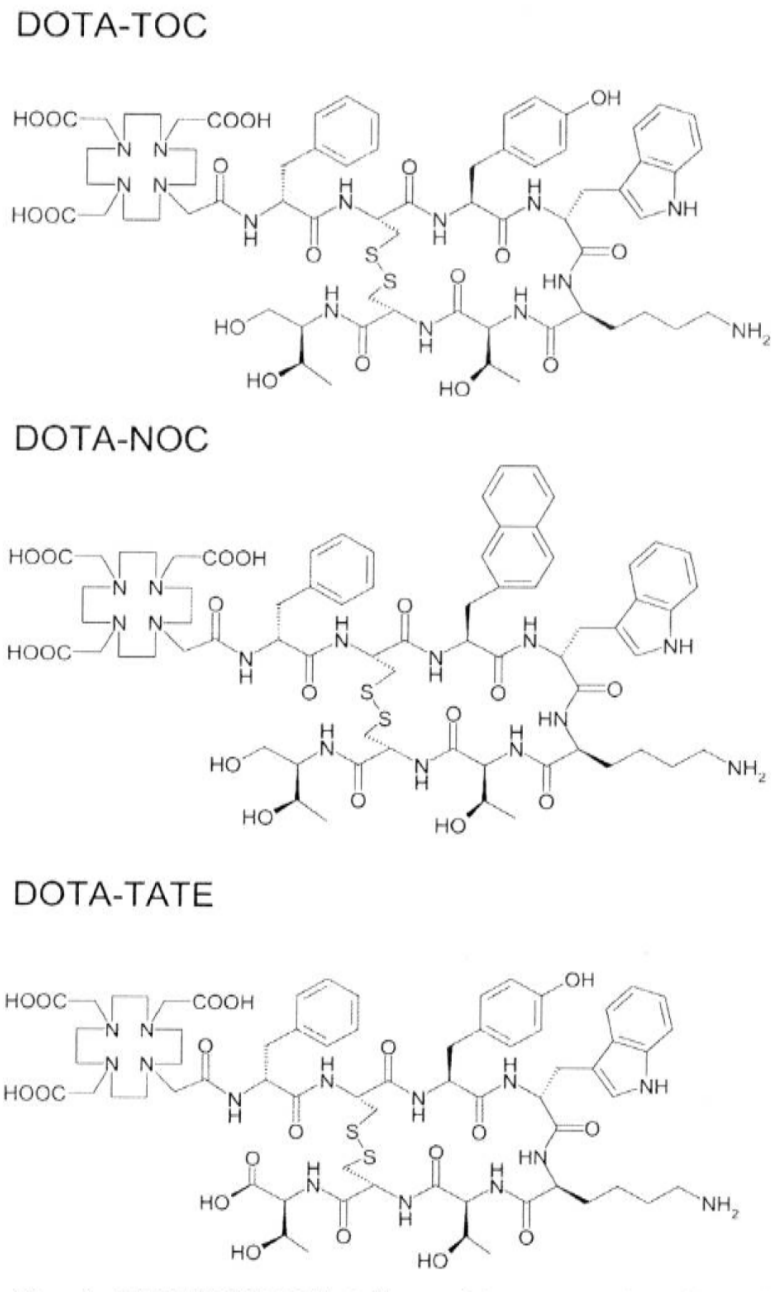


Figure 1.1: Structural formulae of DOTATOC, DOTANOC AND DOTATATE (reproduced from Breeman et al., 2011)

1.3 Gallium chemistry

Gallium is a post-transition metal that is found primarily in the +3 oxidation state. Gallium ions can exist in two oxidation states, Ga^{1+} and Ga^{3+}, but only Ga^{3+} is stable in aqueous conditions. The Ga^{3+} is only stable under acidic conditions and hydrolysis occurs when the pH is increased. At pH higher than 3, the Ga^{3+} forms oxide and hydroxide species which have limited solubility and result in the formation of colloids. Only the dissolved Ga^{3+} is available for complexation with chelators. Hydrolysis should be avoided when producing radiopharmaceuticals. $Ga(OH)_3$ precipitates when the pH is increased but dissolves again as $Ga(OH)_4^-$ between pH 7.4 and 9. The gallate anion $Ga(OH)_4^-$ has a very slow ligand exchange with trans-chelation to other complexing agents (Green and Welch, 1989).

$^{68}Ga^{3+}$ is obtained as $[^{68}Ga]GaCl_3$ from the $^{68}Ge/^{68}Ga$ generator. As discussed above, the pH is very important during radiolabelling with ^{68}Ga due to hydrolysis and forming of insoluble colloids at higher pH. According to Roesch and Riss (2010) ^{68}Ga is labelled to chelators such as DOTA at pH 2.8 to 3.8 in buffered solution where it is present as $[Ga(H_2O)_6]^{3+}$. The most common buffers are acetate, citrate and HEPES. These compounds will form semi-stable complexes with Ga^{3+}, protecting it from hydrolysis to $Ga(OH)_3$. Trans-chelation to the more desired labelling precursor, sometimes with heating, can be achieved at pH values up to 7 (Roesch and Riss 2010). Two other important factors in radiolabelling with ^{68}Ga are the concentration of precursor and the buffer concentration.

1.4 $^{68}Ge/^{68}Ga$ generators

The $^{68}Ge/^{68}Ga$ generator is the main source of ^{68}Ga for routine availability independent from an on-site cyclotron. Advantages of the $^{68}Ge/^{68}Ga$ generator include the potential to prepare multiple radiotracer preparations daily and the use of the same generator over a fairly long period of time (Fani et al., 2008; Maecke et al., 2005). ^{68}Ge is mainly commercially produced in a high energy cyclotron from stable ^{69}Ga via the reaction $^{69}Ga(p,2n)^{68}Ge$ (Roesch, 2012). The ^{68}Ge is isolated from the bombarded gallium target by chemical means and then loaded as a solution onto a column containing a sorbent material where it decays to ^{68}Ga, which can be eluted from the generator. $^{68}Ge/^{68}Ga$ generators contain different sorbent materials for the ^{68}Ge parent radionuclide, such as inorganic substrates such as SiO_2 (Neirinckx and Davis, 1979), TiO_2 (Zhernosekov et al., 2007) and SnO_2 (Aardaneh and Van der Walt, 2006) as well as organic anion exchange resins (Neirinckx and Davis, 1980) and composite sorbents such as nanoceria-polyacrylonitrile (PAN) (Chakravarty et al., 2010). Table 1.1 summarises the most commonly available generators.

Table 1.1: Examples of commercially available $^{68}Ge/^{68}Ga$ generators

Producer	Column Material
Eckert & Ziegler, Berlin, Germany	TiO_2
Cyclotron Co, Obninsk, Russia	TiO_2
iThemba LABS, Somerset West, SA	SnO_2
Isotope Technologies Garching (ITG), Germany	SiO_2/organic
IRE ELiT	Not specified

The TiO_2 and SnO_2 generators are two of the most commonly used commercially available $^{68}Ge/^{68}Ga$ generators. These generators are eluted with aqueous acidic eluents (see 1.3) in order to release the ^{68}Ga daughter radionuclide from the solid support sorbent.

1.5 Eluents used to elute ^{68}Ga eluates from the $^{68}Ge/^{68}Ga$ generators

Due to the different chemical natures of the sorbent materials, eluents with different acidities are required for optimal generator performance in terms of elution profiles and ^{68}Ga yield. This results in eluates with different characteristics such as acidity, which could have an impact on the radiolabelling of DOTA-peptides. Table 1.2 (reproduced from Velikyan, 2015) summarises the various sorbents based on inorganic, organic or mixed materials.

Table 1.2: $^{68}Ge/^{68}Ga$ generator column matrixes and recommended eluents

$^{68}Ge/^{68}Ga$ Generator Column Matrix and Eluents	
Inorganic	
Matrix	**Eluent**
SnO_2	0.6 – 1.0 M HCl
TiO_2	0.1 M HCl
CeO_2	0.02 M HCl
ZrO_2	0.1 M HCl
Zr-Ti ceramic	0.5 M NaOH/KOH; 4 M HCl; acetate; citrate
Nano-zirconia	0.01 M HCl
Organic	
N-methylglucamine	0.1 M HCl; 0.1 M NaOH; citrate; EDTA
Pyrogallol-formaldehyde	0.3 M HCl
Nanoceria-polyacrylonitrile	0.1 M HCl

1.6 Impurities in the ^{68}Ga eluates

1.6.1 Metal contaminants

The presence of metallic impurities in the ^{68}Ga eluate can affect the use of the product by competing with ^{68}Ga in the labelling process, as higher quantities of the ligand are needed and specific radioactivity of the radiolabelled product is reduced (Velikyan, 2015). Some metal cations compete with Ga^{3+} and may form more stable complexes with the same chelator or form colloids and absorb $^{68}Ga^{3+}$, preventing it from complexation (Velikyan, 2015). Gallium metal targets (used in the production of SnO_2 generators) are irradiated and small traces of Ga^{3+} and other chemical non-radioactive impurities from the target components could remain in the final ^{68}Ge solution. The eluate from ^{68}Ga generators contain small quantities of stable Zn^{2+} that is generated from the decay of ^{68}Ga and is a strong competitor in complexation with DOTA-ligands (Velikyan, 2015). Regular elution of the generator reduces the Zn^{2+} concentration (de Blois, et al. 2011). Fe^{3+} is the most critical competitor to Ga^{3+} due to the similar chemistry of these two ions in terms of the complexation reaction and resin adsorption of the tetrachloride anion. Oehlke, et al. (2013) performed studies on the influence of, amongst others, Cu^{2+} and Fe^{3+} on ^{68}Ga-labelling of DOTATATE and found that the metal ion/ligand ratio plays a critical role in labelling efficiency. The European Pharmacopoeia (2013)

specification for gallium (^{68}Ga) chloride solution for Fe and Zn content states that no more than 10 µg of each of these metals should be present per GBq ^{68}Ga in the eluate used for radiolabelling.

1.6.2 ^{68}Ge breakthrough

^{68}Ge breakthrough is also a major safety concern in terms of its presence in the generator eluate because of its long half-life (271 days) that may result in an increased radiation dose to the patient as well as present major challenges in dealing with it as radioactive waste. The European Pharmacopoeia (2013) specification states that no more than 0.001 % of ^{68}Ge should be present in the ^{68}Ga eluate for radiolabelling. Higher breakthroughs have been reported, particularly when the generator was not eluted regularly (de Blois et al., 2011). This is a concern since the ^{68}Ge content of a ^{68}Ga preparation cannot be determined before it is injected into the patient (Decristoforo et al., 2012). The ^{68}Ge in an eluate can only be determined more than 24 h after decay of the ^{68}Ga eluate and then measuring the ^{68}Ga, which is generated due to the decay of the ^{68}Ge present.

1.7 Eluate processing methods and their challenges

Different techniques have been developed for radiolabelling DOTA-conjugated peptides, often using semi- or fully automated synthesis units. The methods are either based on using a fraction of the generator eluate directly for radiolabelling or pre-purification and concentration of the generator eluate using an anion exchange or cation exchange technique.

In order to reduce the influence of metal contaminants that could interfere with radiolabelling, and ^{68}Ge breakthrough, the pre-purification of the ^{68}Ga eluates (anion and cation exchange resin purification) is important for the radiolabelling of DOTA-peptides (Meyer et al., 2003a, 2003b, 2004; Zhernosekov et al., 2007; Velikyan et al., 2008). An added advantage of the pre-purification process is that it reduces the volume of ^{68}Ga solutions, since the labelling at nanomolar peptide concentration levels requires small reaction volumes to maximise labelling yields (Meyer et al., 2003a, 2003b, 2004; Zhernosekov et al., 2007). The time needed to process the generator eluate, and to synthesize and purify the labelled product reduces the overall yields of the product. The fractionation technique (Breeman et al., 2005) has also been used to reduce the volume of the ^{68}Ga eluate.

1.7.1 Anion exchange resin purification

Meyer et al. (2004) described a method in which the ^{68}Ga is adsorbed onto a Bio-Rad AG 1X8 (100 – 200 mesh) anion exchange resin cartridge. Two types of generators were used, the pyrogallol/formaldehyde type (which was eluted with 25 – 30 ml of 5.5 M HCl) and the TiO_2-based generator (which was eluted with 8 ml of 0.1 M HCl). The HCl concentration of the eluates from the generators which are eluted with 0.1 M HCl, had to be adjusted to a concentration of 5.5 M HCl, in order to adsorb the ^{68}Ga as a negatively charged complex on the anion exchange resin. The ^{68}Ga is subsequently rinsed from the anion exchange resin with small volumes of water. The method described by Meyer et al. (2004) separates ^{68}Ge, but the $^{68}Ga^{3+}$ (in 0.1 M HCl) cannot be loaded directly on the anion exchange resin, nor is the Ga^{3+} purified from metallic impurities such as Zn^{2+} and Fe^{3+}. The use of high hydrochloric acid concentrations is a disadvantage, especially in automated systems, because excess acid has to be removed to ensure that the labelling conditions are appropriate.

1.7.2 Cation exchange resin purification

The main step in the cation exchange procedure, as described by, amongst others, Roesch et al. (2006); Zhernosekov et al. (2007) and Asti et al (2008), consists of the transfer of the 0.1 M HCl ^{68}Ga eluate onto a cation exchange resin (e.g. Bio-Rad AG 50W-X8). In these studies, a TiO_2 generator served as the source of ^{68}Ga. The ^{68}Ga is adsorbed on the resin directly from the generator eluate and low volumes of HCl/acetone mixtures are applied to desorb the ^{68}Ga from the resin. More than 97 % of the ^{68}Ga is recovered in the acetone/0.05 M HCl solution (Roesch et al., 2006; Zhernosekov et al. 2007). This cation-exchange purification method results in smaller ^{68}Ga eluate volumes and removes almost all metallic impurities including Ge^{4+}, Ti^{4+}, Zn^{2+} and Fe^{3+}. The purified ^{68}Ga eluate can therefore be used for direct labelling. A disadvantage of this purification method is that it requires the use of acetone as desorption agent, which has to be eliminated completely from the final preparation as acetone is not approved for intravenous use (Kibbe, 2009). Quality control of the final product should therefore include testing for acetone by gas chromatography.

Variations of the Zhernosekov et al. (2007) method were investigated, including combining this method with an anion exchange cartridge (Loktionova et al., 2011) or using a different resin and NaCl solution as an eluent (Mueller et al., 2012). Ocak et al. (2010) performed studies on a TiO_2 generator for the synthesis of ^{68}Ga-labelled DOTA-peptides using Bio-Rad AG 50W-X4 (60 mg) cation exchange resin and Strata-X-C 30 mg cation exchange cartridge

for the purification of the ^{68}Ga eluate. For both resins, more than 90 % of ^{68}Ge was removed and more than 85 % of ^{68}Ga was recovered. The Bio-Rad AG 50W-X4 cation exchanger reduced the Fe, Al and Ti content in the eluate significantly more than the Strata-X-C cation exchange cartridge. They showed that their method could be fully automated and used successfully and reproducibly for the preparation of [^{68}Ga]DOTATOC in high yields. It resulted in a smaller volume of ^{68}Ga eluate and reduced Fe and Zn contaminants. The duration of the process was longer (20 – 25 min compared to 10 min) than the fractionated method of Decristoforo et al. (2007) due to the ^{68}Ga eluate purification on the cation exchanger as well as the additional testing for acetone. The fractionated elution technique however, requires C18 Sep-Pak purification of the labelled DOTA-peptide.

Mueller et al. (2012) described an alternative cation exchange concentration/purification method for ^{68}Ga eluates obtained from a TiO_2 generator. Their method was based on the use of an acidified aqueous sodium chloride (NaCl) solution instead of acetone as the desorption agent. A 5 M NaCl solution containing a small volume of HCl is used to convert the bound ^{68}Ga, which is adsorbed onto a SCX cation exchange cartridge, into the $[^{68}GaCl_4]^-$ species. The $[^{68}GaCl_4]^-$ is then eluted from the SCX cartridge. A labelling method was described that enables high-efficiency labelling of DOTA conjugated peptides in high radiochemical purity (Mueller et al., 2012).

Schultz et al. (2013) adapted the NaCl based method of Mueller et al. (2012) for preparation of ^{68}Ga-labelled radiophamaceuticals for use with an automated system using ^{68}Ga eluates (from a TiO_2 generator) which were purified on a cation exchange cartridge (SCX). Advantages of this method included reduced preparation time (< 15 min) largely due to the omission of organic solvents and not requiring the C18 final purification step.

Martin et al. (2014) investigated purification methods using various (commercial) cation exchange resins and ^{68}Ga eluates from a SnO_2-based generator. These methods to compare the ^{68}Ga-trapping efficiency of the resins as well as elution of the ^{68}Ga from the cartridges were also aimed to reduce the eluate volume and adjust the HCl concentration. Chromafix PS-H^+ gave better results than the other six cation exchange cartridges that were also tested.

Seemann et al. (2015) compared three cation exchange resin based post-processing methods (acetone, ethanol and NaCl), using the TiO_2 generator and the three precursors (DOTATOC, NO2AP^{BP}, and DATA^m). The acetone and ethanol based methods provided greater

reproducibility and yields. For the acetone-based method, the generator was eluted with 5 ml of 0.1 M HCl and ^{68}Ga was trapped on the Bio-Rad AG 50W-X8-400 cation exchange resin. For the ethanol-based method, the generator was also eluted with 5 ml of 0.1 M HCl and ^{68}Ga was trapped on the Bio-Rad AG 50W-X4 cation exchange resin. For the NaCl-based method, ^{68}Ga (in 10 ml of 0.1 M HCl) was trapped on the Merck Lichrolut SCX resin. In the acetone-based method further purification steps are required because the acetone has to be removed. The NaCl method offers the advantage of not using acetone, however, in general it resulted in lower yields as a result of longer labelling times, and changes to the pH and temperature had a large effect on the labelling yield. The ethanol method resulted in labelling yields greater than 98 %

1.7.3 Combined cation-anion exchange resin purification

Müller et al. (2011) investigated the use of a combined cationic and anionic purification method. In this procedure the ^{68}Ga is eluted from the generator in 10 ml 0.1 M HCl. The ^{68}Ga eluate is loaded and adsorbed on a strong cation exchange (SCX) cartridge. A small volume of 5.5 M HCl converts the ^{68}Ga to $[^{68}GaCl_4]^-$ and elutes it from the SCX cartridge. The $[^{68}GaCl_4]^-$ is then adsorbed onto a strong anion exchange (SAX) cartridge. The ^{68}Ga is finally eluted with small quantities of water for the radiolabelling process. Their method however requires two cartridges and the use of 5.5 M HCl during the synthesis.

Loktionova et al. (2011) developed a method for ^{68}Ga eluates from a TiO_2 generator (eluted with 0.1 M HCl) based on processing of the ^{68}Ga eluate on micro resin columns. Pre-concentration and purification of the ^{68}Ga eluate was performed on a cation-exchange resin (50 mg of wet AG 50W-X8), using 80 % acetone/0.15 M HCl solution to remove most of the chemical and radiochemical impurities. The purified ^{68}Ga was then desorbed from the cation exchanger with 4 or 5 M hydrochloric acid solutions. The ^{68}Ga was then loaded on a micro-column containing an anion exchange resin (50 mg of wet AG 1-X8) or 100 mg tetraalkyldiglycolamide-based resin, which allowed direct re-adsorption of ^{68}Ga eluted from the cation exchanger with the high concentration of HCl solution. The ^{68}Ga was finally desorbed from the anion exchange resin with a small volume of water. The aim of their study was to develop a purification technique that combines aspects of the cationic and anionic exchange methods.

Patrascu et al., (2011) performed experiments with an iThemba LABS SnO_2 generator to investigate the purification of the eluate on a cation exchange column (Dowex-50 200 – 400

mesh), an anion exchange column (Dowex-1, 200 – 400 mesh) or both. For the anion separation, the generator was eluted with 1 M HCl and the eluate was adjusted to obtain a 5.5 – 6 M HCl solution, which was loaded onto the anion exchange column. The ^{68}Ga was retained on the column and finally desorbed with water. They found this purification method optimal based on the process time (30 min) and recovered ^{68}Ga (up to 90 %). In the cation separation, the 1.0 M HCl ^{68}Ga eluate was loaded onto the column and the column was washed with different acetone/HCl mixtures. They found this method to be non-reproducible because of the inconsistent recovered ^{68}Ga activity. In the combined anion/cation separation method, the 0.6 M HCl eluate was loaded on the cation exchange column and ^{68}Ga was desorbed from the column with 4 M HCl. The solution was then loaded onto the anion exchange column and recovered in 1 ml water. The combined method offered a good concentration and purification of the eluate, but the process time was too long (40 – 60 min).

1.7.4 Fractionation

The fractionation method uses the fraction of the generator eluate that contains the highest concentration of ^{68}Ga and this fraction is used for radiolabelling (Roesch, 2012). Breeman et al. (2005) found that 80 % of the radioactivity was recovered in 1 ml of the eluate, using fractionated elution. The technique aims to overcome problems associated with the large volume of the eluates (Breeman et al., 2005; de Blois et al., 2011; Rossouw and Breeman, 2012). This may, however, result in a complicated labelling system and loss of activity. This approach also aims to overcome problems with acidic pH, ^{68}Ge content and chemical impurities. The ^{68}Ge and metallic impurities are not removed but are lower because the eluate volume used is lower. Users of this approach have to take care to avoid metallic impurities in the eluate, and purify the radiolabelled peptide after radiolabelling. Contaminants such as ^{68}Ge, which cannot be completely removed with this technique, can be removed with post-labelling purification techniques such as C18 chromatography (Decristoforo et al., 2007). Consistently high labelling efficiencies were obtained using fractionated, un-purified ^{68}Ga eluates from a SnO_2-based generator to label DOTATATE (de Blois et al., 2011; Rossouw and Breeman, 2012).

1.8 General radiolabelling conditions

The literature on the labelling conditions for the radiolabelling of DOTA-peptide ligands is largely based on the TiO_2-based generator. Although the characteristics of the iThemba LABS SnO_2 generator were well described by de Blois et al. (2011) and Sudbrock et al. (2014), additional investigation into aspects of the radiolabelling is required on this generator because of the difference in composition of the ^{68}Ga eluates obtained from these two generators. This is particularly applicable when using eluates in their original matrices (such as fractionated eluates) that have not been pre-processed by means of resin chromatography. For example, in order to achieve optimal elution efficiencies with the SnO_2-based ^{68}Ge/^{68}Ga generator, it has to be eluted with a more acidic eluent as opposed to the 0.1 M HCl required for the TiO_2-based generator. DOTA-peptide labelling is pH-dependent and is conducted at pH values between 3 and 5 (Meyer et al., 2003a, 2004; Breeman et al., 2005). Therefore, when more acidic un-processed ^{68}Ga-containing eluates are used in peptide labelling, labelling methods need to be adapted.

Rossouw and Breeman (2012) investigated a method to prepare ^{68}Ga-labelled DOTATATE using fractionated, un-purified ^{68}Ga eluates by maximising the eluted activity for the labelling and adapting existing labelling methods to accommodate for the large volume and more acidic eluate. Their study describes a rough optimization of the DOTATATE content of labelling mixtures to ensure optimal radiolabelling yields by employing two peptide masses (30 and 50 µg). De Blois et al. (2011) performed DOTATATE-labelling studies with ^{68}Ga eluted from this generator, but with maximum reaction volumes of 1.5 ml. Their study was only performed with DOTATATE and its main focus was to investigate the characteristics of the SnO_2 generator and eluate purification with less focus on radiolabelling. The study by Sudbrock, et al. (2014) on ^{68}Ga-DOTATATE, investigated the influence of several factors on the radiochemical synthesis, including the quantity of DOTATATE, the influence of Fe^{3+} salts and replacement of HEPES buffer with sodium acetate. Their study was only performed with DOTATATE and with peptide masses of 1 – 15 µg in a reaction volume of approximately 2 ml.

1.9 Development of user-friendly kits

In order to improve the user-friendliness of the ^{68}Ga labelling process, there is a need to develop stable kit formulations based on optimized labelling methods. This allows instant preparation of ^{68}Ga-labelled DOTA-peptides within a radiopharmacy environment. By

definition, a kit for radiopharmaceutical preparation contains all reagents required except the radionuclide, in a vial. Production of the radiopharmaceutical preparation may require additional steps such as heating. Kit preparations may be lyophilised (freeze-dried).

Single vial freeze-dried DOTA-peptide kits were developed and evaluated for the preparation of ^{68}Ga-labelled DOTA-peptides (Mukherjee et al., 2014a, 2014c). The $^{68}GaCl_3$ was eluted from a nanoceria-polyacrylonitrile (PAN), composite-sorbent-based ^{68}Ge/^{68}Ga generator using 0.1 M HCl, which was directly added to the kits. Sodium acetate was used as the buffer and a peptide mass of 50 µg was used for the kit formulation. The stability of the kits was investigated over a 4-month period (Mukherjee et al., 2014a).

In another study by the same author (Mukherjee et al., 2014b) freeze-dried DOTATATE kits for radiolabelling with both ^{68}Ga and ^{177}Lu were formulated as a theranostic radiopharmaceutical preparation. They report that the ^{68}Ga was eluted from a SnO_2-based generator with 0.1 M HCl which was loaded on a Strata-X-C cartridge. (According to the literature, 0.6 – 1.0 M HCl is used as the eluent for this generator and the 0.1 M HCl would probably result in a low generator elution yield.) The resin was washed with acetone/0.1 M HCl (80/20) solution to remove the metal impurities. The purified ^{68}Ga was eluted in an acetone/0.02 M HCl (97.6/2.4) solution and directly added to the kit vial. The kits contained DOTATATE quantities of 30, 50 or 200 µg and HEPES buffer. The final preparation was purified by a Sep-Pak C18 cartridge to remove the HEPES and colloids. HEPES buffer was used because ammonium acetate buffer was found to be unstable during the lyophilisation process and sodium acetate buffer was not able to maintain pH 4 after lyophilisation when ^{68}Ga in 0.1 M HCl was used for complexation. The kits were evaluated over a period of 6 months.

A study by Asti et al. (2015) to develop and optimise direct labelling of DOTATOC through a kit-based approach was conducted using eluates from a TiO_2- and a silica-based generator and two synthesis methods. Fractionated 2 ml of 0.1 M HCl ^{68}Ga eluates were added to vials containing 30 µl (1 mg/ml) DOTATOC, sodium acetate buffer and ascorbic acid solution (scavenger). The vial was heated to 100 °C in a heating block for 5 min and the reaction mixture (pH = 3.3) was passed through a C18 cartridge.

DOTA-peptide kits (SomaKit TOC and NETSPOT) have recently been licensed for ^{68}Ga-labelling of somatostatin analogues. SomaKit TOC is used for the preparation of ^{68}Ga-

DOTATOC and NETSPOT is used for the preparation of ^{68}Ga-DOTATATE. These kits consist of one vial of lyophilized powder (containing 40 µg edotreotide/DOTATOC or DOTATATE and excipients) and one vial containing a buffer. The generator is eluted directly into the vial containing the peptide, the buffer is added and the vial is heated at 95 °C for no more than 10 minutes, after which it is removed from the heat and left to cool at room temperature for about 10 minutes. Eluates from the GalliaPharm generator (0.1 M HCl) by Eckert & Ziegler Radiopharma GmbH are compatible with both SomaKit TOC and NETSPOT. Although the cost of these licensed kits may be higher than any unlicensed kits, the major advantage of using licensed kits is that they have passed all the required preclinical and clinical tests to ensure patient safety and they are produced according to GMP specifications. These kits make the compounds available to PET centres that may not have the necessary infrastructure or capacity to prepare their own kits.

1.10 European Pharmacopoeia specifications

The following specifications are prescribed in the European Pharmacopoeia 8.0 (2013):

1.10.1 Gallium (^{68}Ga) chloride solution for radiolabelling

Appearance:	Clear and colourless solution
Half-life:	62 to 74 min
Radionuclidic identity (gamma-ray spectrometry):	0.511 MeV and 1.077 MeV
Radionuclidic purity (gamma-ray spectrometry):	≥ 99.9 %
Germanium-68:	≤ 0.001 %
Radiochemical purity (TLC):	≥ 95 %
pH:	≤ 2
Iron:	≤ 10 µg/GBq
Zinc:	≤ 10 µg/GBq
Bacterial endotoxins:	< 175 IU/V (where v is the maximum volume to be injected)

1.10.2 Gallium (^{68}Ga) Edotreotide injection (DOTATOC)

Appearance:	Clear and colourless solution
Half-life:	62 to 74 min
Radionuclidic purity (gamma-ray spectrometry):	≥ 99.9 %
Germanium-68:	≤ 0.001 %
Radiochemical purity:	≥ 91 %
pH:	4.0 to 8.0
Bacterial endotoxins:	< 175 IU/V (where v is the maximum volume to be injected)
Sterility:	Sterile

Problem Statement

Based on the literature, extensive radiolabelling studies have been performed on the TiO_2 generator, however labelling studies using ^{68}Ga eluates from the SnO_2-based $^{68}Ge/^{68}Ga$ generator are relatively limited and need to be further investigated. One of the important aspects is the high acidity of the eluates, which affects the radiolabelling process. An eluent with lower acidity but similar elution capacity would be preferred. Another aspect that requires investigation is ^{68}Ge breakthrough and finding the most efficient way to eliminate its presence in the final radiopharmaceutical product. Metal contaminants and the large volume of the ^{68}Ga eluate also need further investigation. In addition, a more thorough optimization of parameters such as DOTA-peptide content of labelling mixtures and post labelling purification techniques, using all three DOTA-peptides, is required. The labelling process needs to be optimised with real-life constraints within the clinical and research environment (such as the infrequent use of the generator) and improved user-friendliness, such as the development and the use of labelling kit formulations. Furthermore, the purification and processing of eluates prior to labelling warrant additional investigation.

Chapter 2: Aim and Objectives

2.1 Aim

The aim of the study was to investigate the effect of various parameters on the radiolabelling of somatostatin DOTA-peptide derivatives with ^{68}Ga eluted from a tin dioxide (SnO_2)-based ^{68}Ge/^{68}Ga generator, using differently processed ^{68}Ga eluates.

2.2 Objectives

Different ^{68}Ge/^{68}Ga generator elution and eluate processing techniques exist. The initial objective of this study was to present radiolabelling optimization parameters, using ^{68}Ga eluates processed with different techniques. It was anticipated that the final results of this study would indicate which of the eluate processing techniques is the most efficient in terms of radiolabelling yields and radiochemical purities of labelled products. This study aimed to highlight all probable pitfalls that could be associated with the radiolabelling of DOTA-peptides, using ^{68}Ga eluates from a SnO_2-based generator, as well as finding efficient ways to eliminate them. It should result in the development of reliable and user-friendly procedures to obtain ^{68}Ga-labelled peptides of high radiopharmaceutical quality. The objectives of this study are listed below:

2.2.1 To investigate elution of the ^{68}Ge/^{68}Ga generator with different eluents in order to obtain elution data across a wide range of eluent acidity:

- Investigation of elution of the SnO_2-based generator with alternative eluents such as the more acidic 0.8 M hydrochloric acid (HCl) and 1.0 M HCl solutions as well as the less acidic 0.4 M HCl and 0.2 M HCl solutions.
- Comparison of elution efficiencies, ^{68}Ge breakthrough and the content of various metals in eluates.

2.2.2 To optimise radiolabelling parameters using the fractionated elution method:

The work is based on the post-labelling purification method of Rossouw and Breeman (2012), but aimed to further optimize and adjust methods according to the requirements for other DOTA-peptides. In addition to DOTATATE, this study was also planned to include other important DOTA-peptides such as DOTATOC and DOTANOC and involved a more comprehensive investigation of the influence of peptide mass on labelling (15 to 50 μg) in a fixed reaction volume of approximately 2.6 ml. The following aspects were investigated:

- Optimization of the peptide (DOTATATE, DOTATOC and DOTANOC) content of radiolabelling formulations, using fractionated ^{68}Ga eluates, with the aim to obtain optimal radiolabelling efficiencies and radiochemical yields of radiolabelled peptides. In order to obtain optimal radiochemical yields for all the DOTA-peptides, the Sep-Pak C18 purification technique of the labelled peptides was optimized by using varying eluent mixtures for final elution of the product;
- Comparison of the heating capabilities of two different heating modes used in the radiolabelling process (a water-bath versus a heating block) and investigation of the effect on the radiolabelling efficiency and radiochemical yield; and
- Determination of the influence of varying periods up to 21 days of non-elution of the ^{68}Ge/^{68}Ga generator (e.g. after weekends, long weekends, maintenance periods) on radiolabelling results as well as on the metal ion content of the ^{68}Ga eluates.

2.2.3 To develop and evaluate user-friendly single vial DOTA-peptide kit formulations, using the fractionated elution method:

- Development of single vial DOTA-peptide kit formulations based on the optimized labelling conditions using the fractionated elution method. These kits containing the peptide and buffer were aimed at improving the convenience and user-friendliness of the labelling procedure.
- Evaluation of the long-term stability of the single vial DOTA-peptide kits, as reflected by the labelling results; and
- Comparison between the use of DOTA-peptide single vial kits versus the use of DOTA-peptide stock solutions (using the fractionated elution method).

2.2.4 To investigate the concentration and purification of ^{68}Ga eluates on different cation exchange resins.

2.2.5 To purify various concentrations of metal-spiked (Ga, Zn, Fe and Cu) ^{68}Ga eluates on a cation exchange resin.

2.2.6 To optimize the radiolabelling of DOTATATE, using ^{68}Ga eluates that have been pre-concentrated on a suitable cation exchange resin cartridge.

2.2.7 To develop and evaluate user-friendly single vial DOTATATE kit formulations, using the cation exchange resin-processed ^{68}Ga eluates.

Chapter 3: Study Design and General Methods

The study mainly involved an investigation into the radiolabelling of three peptide ligands with ^{68}Ga eluted from the SnO_2-based generator developed at iThemba LABS. The selected ligands were the somatostatin derivatives 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid-D-Phe^1-Tyr^3-octreotate (DOTATATE), 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid-D-Phe^1-Tyr^3-octreotide (DOTATOC) and 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid-1-Nal3-Octreotide (DOTANOC).

Initially, various elution characteristics of a SnO_2-based generator with different concentrations of HCl were investigated. The next phase of the study involved investigations into the use of differently processed ^{68}Ga eluates (fractionated and cation resin purified) in radiolabelling. Initially these investigations involved the optimization of radiolabelling parameters, using fractionated eluates, followed by an investigation into the improvement of the user-friendliness of the labelling procedure, using kit formulations. The latter part covered the processing/purification of eluates on various types of cation exchange resins followed by the selection of the most efficient resin and the use of the processed eluates emanating from this resin in conventional labelling and eventually in kit labelling. The ability of this resin to remove metals, which were deliberately introduced into eluates, was also investigated.

3.1 General

All experiments were repeated for a minimum number of three times, as well as on more than one generator and different batches of peptides to confirm repeatability of results. At least three SnO_2 ^{68}Ge/^{68}Ga generators, which were produced by and obtained from iThemba LABS (Somerset West, South Africa), were used. The number of experiments and generators decided upon were to ensure reliable results but limit the radiation exposure to the investigator. Radiolabelling efficiency and radiochemical purity were determined by means of high performance liquid chromatography (HPLC) and instant thin layer chromatography (iTLC). All ^{68}Ga activity data are expressed as decay corrected. Sterility and endotoxin testing were performed on DOTA-peptide kits. All quality control methods were validated and some of these validations formed part of another study (Davids, 2017). All chemicals used were of analytical grade.

The researcher is a registered radiation worker. The radiation exposure (finger and whole-body doses) was monitored and recorded with the required dosimetry (electronic personal

dosimeter, finger rings and thermoluminescent dosimeter). The radioactive waste produced was handled according to iThemba LABS' radiation safety policies and procedures. No human studies were conducted as part of this research project. This study was approved by the Stellenbosch University Health Research Ethics Committee (Approval no. S15/09/214) and permission was granted for the experimental work to be conducted at iThemba LABS.

3.2 Equipment

Radioisotope Dose Calibrator:	Capintec CRC-55tR
Semi-micro Balance:	Mettler Toledo XP105DR
Radio-TLC scanner:	Carroll & Ramsey Omni-rad EZ Scan
Incubator (32.5 ºC):	Labcon FSIM16
Incubator (22.5 ºC):	Labcon LTIM10
Endotoxin Testing System:	
Microplate reader:	Bio-Tek ELx800
Microplate Incubator/Shaker:	BMG THERMOstar
HPLC System:	
Binary HPLC Pump:	Perkin Elmer Series S200
HPLC Injector:	Rheodyne model 7125
HPLC column:	Phenomenex Luna C18 (250 X 4.6mm, 5µm)
Integrator:	Shimadzu Chromatopac C-R8A
HPLC detector:	RadiationNa(Tl) radiation flow detector coupled to a high voltage power supply and ratemeter (Ortec)
Gamma Spectrometer System:	
Genie 2000 software:	Canberra
Germanium detector:	DSG Detector Systems GmbH
Inductively coupled plasma optical emission spectrometer (ICP-OES):	Horiba Jobin Yvon Ultima
Freeze dryer:	Edwards Freeze dryer Modulyo
Vacuum Pump:	N 726 FT.18 Vacuum Pump
Vortex mixer:	Vortex – Genie 2 G560E

3.3 Solutions

3.3.1 0.6 M HCl Eluent

A volume of 63.41 ml of 30 % hydrochloric acid (HCl) Suprapur (9.642 M), catalogue number 1.00318.0025, (Merck, Darmstadt, Germany) was diluted with ultra-pure water and made up to 1000 ml 0.6 M HCl.

3.3.2 Stock solutions of the three DOTA-peptide ligands

The DOTA-peptides (DOTATATE, DOTATOC, and DOTANOC) were purchased from JPT Peptide Technologies GmbH (Berlin, Germany). 1 mg/ml DOTA-peptide stock solutions were prepared using ultra-pure pharmaceutical grade water and stored at -20 °C. Frozen stock solutions were thawed to room temperature before being used in radiolabelling. These frozen stock solutions were frozen and thawed for a total number of cycles of up to 10 times.

3.3.3 HPLC Mobile Phases

HPLC mobile phase A:

0.1 % (w/v) trifluoracetic acid (TFA)

A volume of 0.67 ml of TFA, catalogue number 302031, (Sigma-Aldrich, St. Louis, Missouri, United States) was made up to 1000 ml with ultra-pure water.

HPLC mobile phase B:

Acetonitrile, catalogue number 34851, (Sigma-Aldrich, St. Louis, Missouri, United States**)**

3.3.4 TLC Mobile Phase: 1 M ammonium acetate:methanol (1:1)

77.08 g of ammonium acetate, catalogue number 431311, (Sigma-Aldrich, St. Louis, Missouri, United States) was weighed and diluted to 1 litre using ultra-pure water. A 1:1 solution of ammonium acetate:methanol was prepared by adding equal volumes of 1 M ammonium acetate solution and methanol, catalogue number 34860 (Sigma-Aldrich, St. Louis, Missouri, United States**).**

3.3.5 ICP standard solutions (1 ppm, 5 ppm and 10 ppm) of zinc (Zn), iron (Fe), tin (Sn), copper (Cu), gallium (Ga) and aluminium (Al)

1000 ppm standards solutions (catalogue numbers: Zn in 5 % HNO_3 – 88118, Fe in 5 % HNO_3 – 88073, Sn in 20 % HCl – 88112, Cu in 5 % HNO_3 – 88061, Ga in 5 % HNO_3 / trace (tr) HCl – 88066 and Al in 5 % HCl – 33557) were purchased from Alfa Aesar (Haverhill,

Massachusetts, United States) and diluted to 100 ppm with 0.6 M HCl before using to prepare 1 ppm, 5 ppm and 10 ppm solutions of each metal.

3.3.6 Tryptic soy broth (TSB) for sterility testing

15 g of TSB powder, catalogue number 22092, (Sigma-Aldrich, St. Louis, Missouri, United States) was dissolved in 500 ml of ultra-pure water, heated with stirring and boiled for 1 min. This solution was transferred to storage containers and was sterilized by autoclaving at 121 ºC for 15 min.

3.3.7 Thioglycollate broth (TB) for sterility testing

14.5 g of TB, catalogue number 70157, (Sigma-Aldrich, St. Louis, Missouri, United States) was dissolved in 500 ml of ultra-pure water, boiled until the TB was completely dissolved and transferred to storage containers. It was then sterilized by autoclaving at 121 ºC for 15 min.

3.3.8 2.5 M sodium acetate solution (NaOAc)

A 2.5 M NaOAc solution was prepared by dissolving 34 g of sodium acetate trihydrate salt, catalogue number SAAR5821010EM, (Merck, Darmstadt, Germany) in 100 ml ultra-pure pharmaceutical grade water.

3.3.9 5.5 M HCl

A 5.5 M HCl was prepared by making up 290.70 ml of 30 % HCl Suprapur (9.642 M), catalogue number 1.00318.0025, (Merck, Darmstadt, Germany) to 500 ml with ultra-pure pharmaceutical grade water.

3.3.10 5 M NaCl / 5.5 M HCl 40:1 (v/v)

5 M NaCl was prepared by dissolving 29.22 g of NaCl salt, catalogue number 31434 (Riedel-de Haën, Honeywell, Morris Plains, New Jersey, United States) in 100 ml water.
The 5 M NaCl / 5.5 M HCl 40:1 (v/v) solution was prepared by mixing 10 ml of 5 M NaCl and 0.25 ml of 5.5 M HCl.

3.4 General Methods

3.4.1 Elution of the generator

The SnO_2-based $^{68}Ge/^{68}Ga$ generator (double-loaded with ^{68}Ge activity) was supplied by iThemba LABS (Somerset West, South Africa). The generator has an inlet and outlet polyethylene tube with luer fittings. A sterile polyethylene 10 ml syringe is attached to the inlet tube via the luer fitting to elute the ^{68}Ga from the generator with 0.6 M HCl (except when different eluents were investigated) (refer to 3.3.1 for preparation of the eluent). The generator was eluted (for up to 1 year) daily on weekdays, except when the experiments were performed to assess the effects of various periods of non-elution of the generator on the radiolabelling results. All ^{68}Ga activities were measured using a Capintec Dose Calibrator CRC-55tR (New Jersey, United States).

3.4.2 Sep-Pak C18 post-radiolabelling purification

The radiolabelled mixtures were loaded on a 500 mg, 3 cc, Sep-Pak C18 cartridge, catalogue number WAT036815, (Waters, Milford, Massachusetts, USA) which was attached to a needle that had been pushed through the centre of a rubber stopper sealing a glass vial. A second needle was connected to a syringe via polyethylene tubing and was also pushed though the stopper. The Sep-Pak C18 cartridge had been pre-conditioned with 4 ml absolute ethanol, catalogue number 32221, (Sigma-Aldrich, St. Louis, Missouri, United States) followed by 2 ml deionised water. Elution was conducted at a rate of approximately 1 – 1.5 ml/min and was controlled with a syringe plunger. The empty reaction vial was rinsed with 2 ml 0.9 % sodium chloride solution (Fresenius Kabi, Port Elizabeth, South Africa), which was then transferred onto the cartridge and eluted. The Sep-Pak C18 cartridge was eluted with a further 6 ml 0.9 % sodium chloride solution. During both these steps, the cartridge was not completely drained. The collection vial (**S1**) was then removed and replaced with another vial (**S2**). The reaction vial was rinsed with 0.7 ml ethanol:saline mixtures (50:50 unless specified otherwise), which was then transferred to the Sep-Pak C18 cartridge and used to desorb the labelled peptide. The cartridge was not completely drained and 2.25 ml 0.9 % sodium chloride solution was transferred to the C18 cartridge to rinse. The retained ethanol:saline mixture on the cartridge from the previous elution step was displaced and the cartridge was completely drained to remove any retained ethanol (C18 cartridge dead volume ≅ 0.6 ml). The measured desorbed activity in **S2** was expressed as the radiochemical yield of labelled peptide.

3.4.3 HPLC Analysis

Refer to section 3.2 for the HPLC equipment.

The mobile phases were:

Mobile phase A: 0.1 % (m/v) TFA

Mobile phase B: Acetonitrile

A sample with a maximum volume of 50 µl (depending on the radioactivity concentration) was taken from the pre-purified and post-purified ^{68}Ga-DOTA-peptide solutions for HPLC analysis.

Elution was carried out at a flow rate of 1 ml/min, using the following elution programme: 0 – 2 min (100 % A); 2 – 12 min (70 % B); 12 – 15 min (100 % B). Under these conditions free ^{68}Ga eluted at 2.6 – 3.7 min, small traces of radiochemical impurities, which were occasionally present but not quantifiable, eluted at 6.3 – 9.3 min, while [^{68}Ga]DOTATATE eluted at 12.2 – 12.8 min, [^{68}Ga]DOTATOC eluted at 12.2 – 12.7 min and [^{68}Ga]DOTANOC eluted at 12.7 – 13.2 min. See Annex 1 for examples of chromatograms.

3.4.4 Sterility Testing

Sampling for sterility was performed in a Class A laminar airflow unit. Each sample was tested using soybean-casein digest medium for aerobic bacteria and fungi, and fluid thioglycollate medium for anaerobic bacteria. The volume of the sample did not exceed 10 % of the volume of the medium. The soybean-casein digest medium and fluid thioglycollate medium were added to the samples aseptically. The sample containing soybean-casein digest medium was incubated at 22.5 ± 2.5 °C and the sample containing fluid thioglycollate medium was incubated at 32.5 ± 2.5 °C. A positive *E. Coli* sample (*E. Coli* + broth only) plus a negative sample were incubated together with the other samples. Samples were incubated for two weeks and checked on a daily basis for appearance of growth. The appearance of the media would have changed from clear to cloudy if bacterial growth was present.

3.4.5 Endotoxin Testing

The chromogenic method was used to test for endotoxins. A LAL Test Chromogenic kit, catalogue number 50-647U (Lonza, Basel, Switzerland) was used which contained the endotoxin stock solution, LAL reagent, substrate and LAL reagent water. The LAL reagent water was used to reconstitute the endotoxin stock solution, the LAL reagent and substrate. A set of endotoxin standards, ranging from 0.1 international units per millilitre (IU/ml) to 1.0 IU/ml, was prepared using the endotoxin stock solution. The pH of the samples was adjusted

to pH 6.0 – 8.0 with 0.1 N sodium hydroxide (NaOH) prepared from 1 N NaOH titrisol, catalogue number 1.09956.0001, (Merck, Darmstadt, Germany) and LAL reagent water. The absorbance was determined with a BioTek ELx800 plate reader (BioTek, Winooski, United States) and was plotted against the concentration of the standards to create a calibration curve. The calibration curve was used to determine the concentration of endotoxins in the samples. The maximum dilution volume was determined to be 10. An endotoxin standard (0.25 IU/ml) was used as a positive control and the negative control contained LAL reagent water instead of the endotoxin standard. At this dilution volume and pH there was no inhibition of the LAL reaction.

3.4.6 Determination of ^{68}Ge Breakthrough in eluates and ^{68}Ga-labelled preparations

The ^{68}Ge breakthrough was determined at least 24 hours after elution or preparation of the labelled product in order to allow all ^{68}Ga in the initial sample to decay, so that any ^{68}Ga present was due to the decay of ^{68}Ge. A Canberra gamma spectrometer with a germanium detector and Genie 2000 software was used. A standard solution was prepared by diluting 1.11 MBq of ^{68}Ge solution to a volume of 10 ml with 0.6 M HCl. The ^{68}Ge in the sample was quantified using the 511 keV peak (of the ^{68}Ga). Counts on the gamma spectrometer were performed for 1000 seconds each and spectra of the sample, standard solution and background were obtained. The breakthrough (expressed as a percentage) was defined as the ratio of activity of ^{68}Ge divided by the initial ^{68}Ga activity.

3.4.7 Determination of Metal Contaminants

The metal contaminants in the samples were determined using a Horiba Jobin Yvon Ultima inductively coupled plasma optical emission spectrometer (ICP-OES) (Jobin-Yvon, Paris, France). The concentration of metals in the samples was determined from calibration curves ranging from 1 ppm to 25 ppm.

3.4.8 Determination of elution efficiency (yield)

The elution efficiency (yield) was determined as follows:
The generator was eluted with a total of 10 ml eluent and the activity in the ^{68}Ga eluate was measured in a dose calibrator. The elution efficiency was based on the nominal ^{68}Ge activity on the generator column and calculated in the following way:

$$\text{Elution Efficiency (\%)} = \frac{^{68}\text{Ga activity at time of elution x 100}}{^{68}\text{Ge activity (nominal) on the column at the time of elution}}$$

3.4.9 Statistical Analysis

The Univariate Tests of Significance were used to analyse the different effects. Where the p-value was found to be significant ($p < 0.05$), further statistical tests (LS Means test, LSD test and weighted means) were performed.

3.4.10 Concentration of ^{68}Ga eluates on a cation exchange resin

A cation exchange resin cartridge was pre-conditioned by eluting with 1 ml 5.5M HCl, followed by 5 ml deionised water at an elution rate of approximately 1 – 1.5 ml/min by applying a negative pressure inside the vial. The vial was replaced with a 10 ml borosilicate vial and its content was discarded. The ^{68}Ga eluate was loaded onto the pre-conditioned cartridge and eluted into the waste vial (**Fr1**). The resin was run dry and eluted with 1 ml water into the same waste vial (**Fr1**). The vial was sealed and replaced with another vial. The cartridge was loaded with 0.5 ml of a mixture of 5 M NaCl and 5.5 M HCl 40:1 (v/v) and the ^{68}Ga was eluted from the resin. The resin was run dry, the vial (**Fr2**) sealed and the activities of both vials (**Fr1** and **Fr2**), as well as the residual activity in the cartridge, were determined.

Chapter 4: Investigation of elution of the $^{68}Ge/^{68}Ga$ generator with different hydrochloric acid concentrations

4.1 Introduction

Due to the different chemical natures of sorbent materials, eluents with different acidities are required for optimal generator performance in terms of elution profiles and ^{68}Ga yield. A SnO_2-based generator requires higher acid concentrations than a TiO_2-based generator (Aardaneh and Van der Walt, 2006; de Blois et al., 2011; Decristoforo et al., 2012; Sudbrock et al., 2014; Velikyan, 2015). Studies on the use of the SnO_2-based generator go back as far as 1980. A study by Loc'h et al. (1980), using a SnO_2-based generator, found that the higher acidity of the eluent improves the ^{68}Ga elution yield but resulted in greater ^{68}Ge contamination. The authors used HCl concentrations of 1 to 8 M in their investigations, but eventually resorted to using 1 M HCl as the most suitable compromise. Aardaneh and Van der Walt (2006) used 1 M and 4 M HCl solutions for elution of the iThemba LABS SnO_2 generator initially to compare with Loc'h et al (1980). De Blois et al. (2011) investigated iThemba LABS SnO_2 generators in HCl ranges of 0.3 – 1.0 M and ^{68}Ga elution yields were established. Das et al. (2013) eluted their in-house prepared SnO_2 generator with 1.0 M HCl. Rossouw and Breeman (2012) eluted a more than 12-month old iThemba LABS SnO_2 generator with 0.6 M HCl.

The manufacturer recommends that the iThemba LABS SnO_2-based generator should be eluted with 0.6 M HCl compared to the 0.1 M HCl used for the TiO_2-based generator to achieve optimal elution yields (^{68}Ga activity at time of elution, expressed as a percentage of the nominal ^{68}Ge activity on the column at the time of elution). There is, however, to the author's knowledge, only limited published data available on the characteristics of eluates (only elution efficiency/yields) resulting from the elution of a SnO_2-based generator with eluent concentrations lower than 0.6 M HCl.

4.2 Aim

The purpose of this investigation was to elute the SnO_2-based generator with various concentrations of HCl (0.2 M, 0.4 M, 0,6 M, 0.8 M and 1.0 M) in order to obtain and compare data on the elution efficiencies, ^{68}Ge breakthrough and metal contaminants of the ^{68}Ga eluates using each of these eluents and to qualify the "possible benefits over the risks" of using any particular eluent over the recommended 0.6 M HCl.

4.3 Materials and Methods

General methods are summarised in chapter 3. This section describes variations and details specific to the work described in this chapter.

4.3.1 Preparation of eluents

The HCl solutions (0.2 M – 1.0 M) were prepared as follows:

0.2 M HCl:

A volume of 21.14 ml of 30 % HCl was diluted with ultra-pure water and made up to 1000 ml 0.2 M HCl.

0.4 M HCl:

A volume of 42.27 ml of 30 % HCl was diluted with ultra-pure water and made up to 1000 ml 0.4 M HCl.

0.6 M HCl:

A volume of 63.41 ml of 30 % hydrochloric acid (HCl) Suprapur (9.642 M), catalogue number 1.00318.0025, (Merck, Darmstadt, Germany) was diluted with ultra-pure water and made up to 1000 ml 0.6 M HCl.

0.8 M HCl:

A volume of 84.55 ml of 30 % HCl was diluted with ultra-pure water and made up to 1000 ml 0.8 M HCl.

1.0 M HCl:

A volume of 105.7 ml of 30 % HCl was diluted with ultra-pure water and made up to 1000 ml 1.0 M HCl.

4.3.2 Elution of the generator and determination of elution profiles

^{68}Ga was eluted from a 1 to 2- month old 1110 MBq (double-loaded with 2220 MBq ^{68}Ge) SnO_2-based ^{68}Ge/^{68}Ga generator up to thrice daily on weekdays, allowing at least 5 hours between each elution. The generator was eluted at least 6 times in total with 10 ml of each concentration of HCl (in the following sequence: 0.6 M, 0.4 M, 0.2 M, 0.8 M and 1.0 M) with rinses of 10 ml between each concentration of HCl. The generator was eluted with 0.5 ml fractions of the different HCl concentrations up to a volume of 10 ml to construct elution profiles for each eluent.

4.3.3 Determination of the elution efficiency (yield)

The terms elution efficiency and elution yield can be used interchangeably and hereafter, the term elution yield will be used. The generator was eluted with a total of 10 ml eluent (see 4.3.1 for preparation of eluents) and the activity in the ^{68}Ga eluate was measured in a dose calibrator. This data were used to determine the elution yield of the generator. The elution yield was based on the nominal ^{68}Ge activity on the generator column (1110 MBq) and calculated as in 3.4.8.

4.3.4 Determination of ^{68}Ge breakthrough

The ^{68}Ge breakthrough was determined in each sample as described in 3.4.6. The same samples were used hereafter to determine the concentration of metals in each sample.

4.3.5 Determination of metal contaminants

Standard solutions (1 ppm, 5 ppm and 10 ppm) of zinc (Zn), iron (Fe), copper (Cu) and gallium (Ga) were prepared as in 3.3.5. These standards were used to determine the metal contaminants in the samples by means of the Horiba Jobin Yvon Ultima inductively coupled plasma optical emission spectrometer (ICP-OES) (see 3.4.7).

4.4 Results

The results in Table 4.1 represent data on the effect of eluents containing different HCl concentrations on generator elution yield, ^{68}Ge breakthrough and metal contaminants.

Table 4.1: Effect of different eluents on generator elution yield, ^{68}Ge breakthrough and metal contaminants

HCl Conc.	Elution Effic. (%)	^{68}Ge break-through (%)	Metals (ppm)				
			Fe	Zn	Ga	Cu	Total
0.2 M (n=4)	18 ± 0.6	0.004 ± 0.001	0.32 ± 0.02	0.42 ± 0.13	0.15 ± 0.03	0	0.89
0.4 M (n=4)	66 ± 2	0.008 ± 0.003	0.34 ± 0.01	1.35 ± 0.08	0.17 ± 0.01	0	1.86
0.6 M (n=4)	99 ± 3	0.008 ± 0.003	0.39 ± 0.01	1.55 ± 0.01	0.16 ± 0.01	0	2.10
0.8 M (n=4)	118 ± 3	0.591 ± 0.06	0.23 ± 0.06	1.67 ± 0.41	0.40 ± 0.03	0	2.30
1.0 M (n=4)	126 ± 3	0.620 ± 0.02	0.34 ± 0.05	2.31 ± 0.9	0.47 ± 0.1	0.03 ± 0.02	3.15

4.4.1 *Determination of the elution profiles for the different eluents*

Figures 4.1 – 4.5 represent the elution profiles of ^{68}Ga obtained when eluting a 1 – 2-month old generator with different concentrations of HCl (0.2 M – 1.0 M) as eluents.

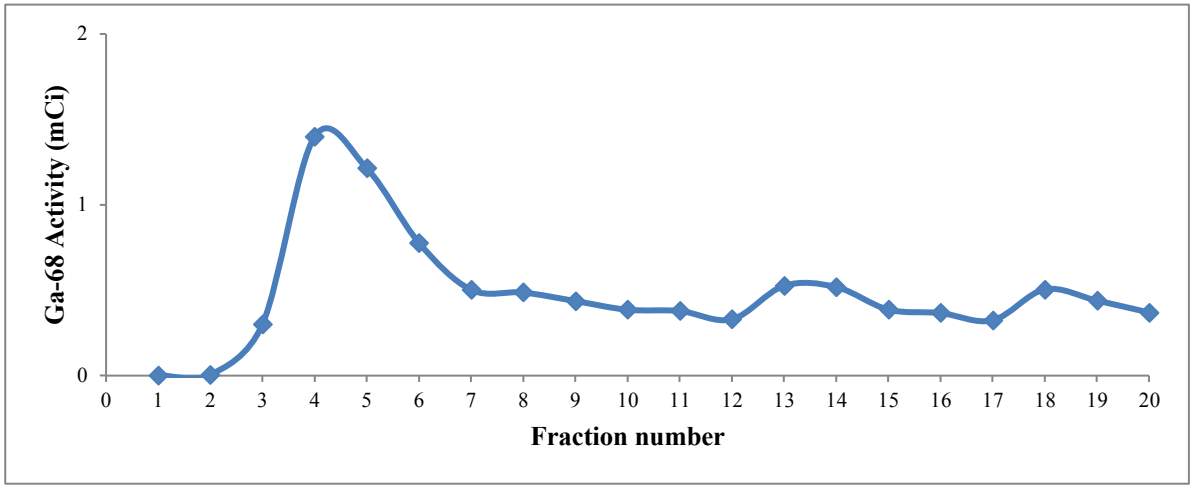


Figure 4.1: Elution profile of ^{68}Ge/^{68}Ga generator using 0.2 M HCl as eluent

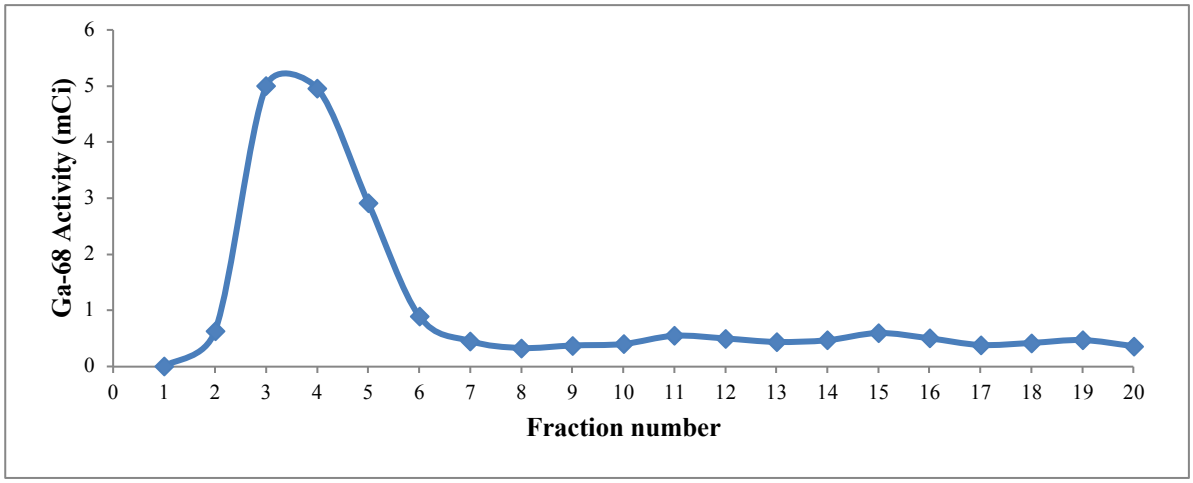


Figure 4.2: Elution profile of ^{68}Ge/^{68}Ga generator using 0.4 M HCl as eluent

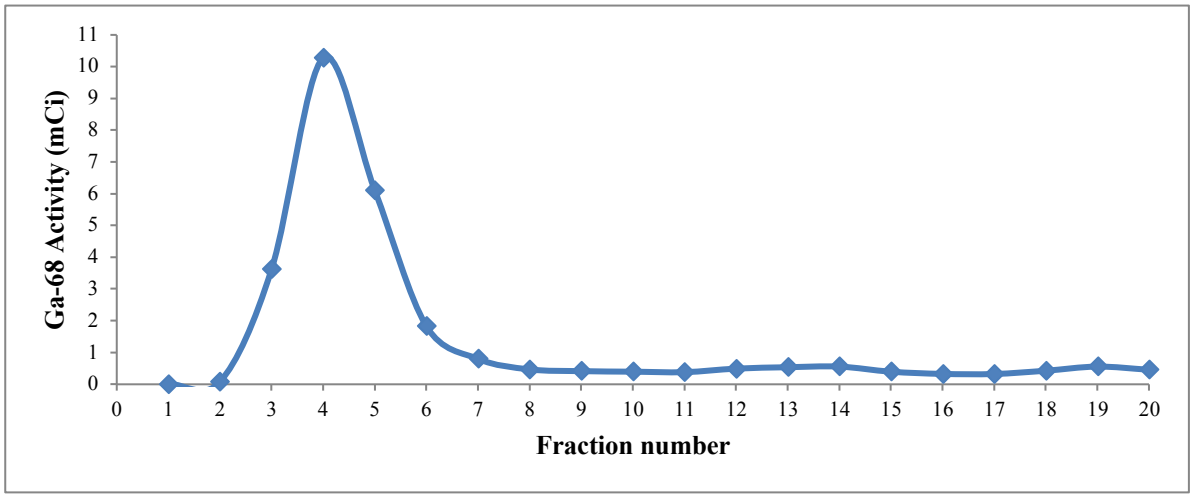


Figure 4.3: Elution profile of ^{68}Ge/^{68}Ga generator using 0.6 M HCl as eluent

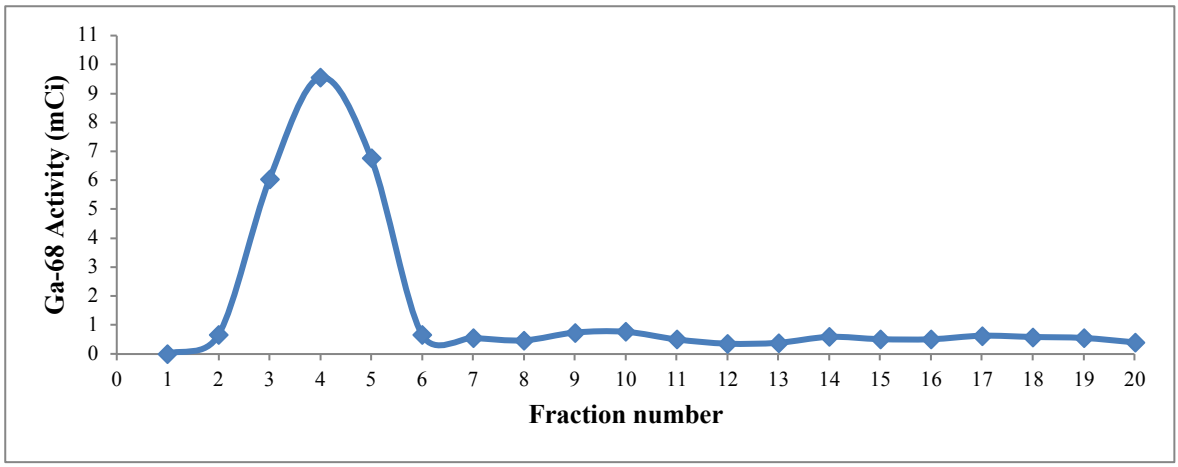


Figure 4.4: Elution profile of $^{68}Ge/^{68}Ga$ generator using 0.8 M HCl as eluent

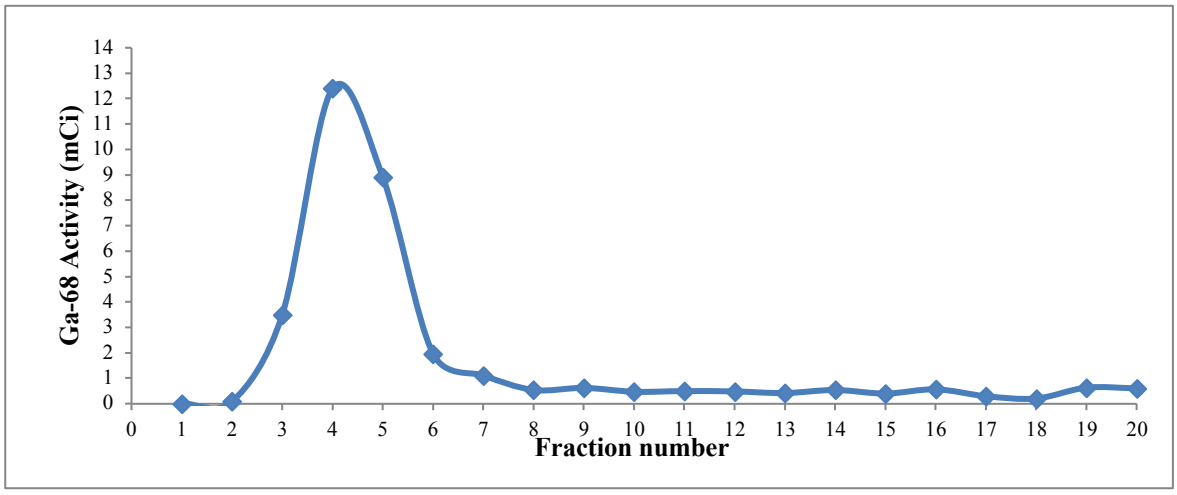


Figure 4.5: Elution profile of $^{68}Ge/^{68}Ga$ generator using 1.0 M HCl as eluent

Figure 4.6 below represents the combined elution profiles of the various eluents containing different concentrations of HCl.

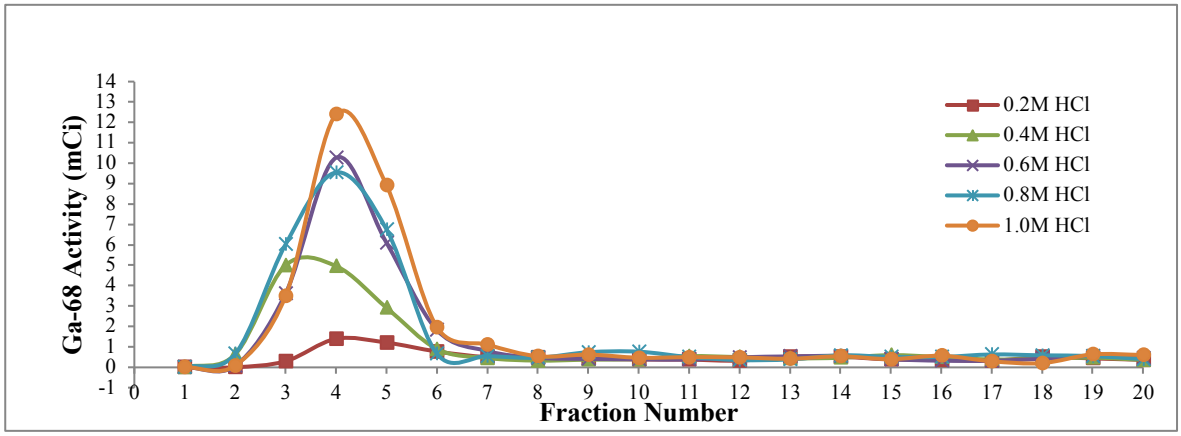


Figure 4.6: Combined elution profiles of $^{68}Ge/^{68}Ga$ generator using various HCl concentrations as eluents

4.4.2 Determination of the elution efficiency (yield)

The elution yield of the generator increased steadily with an increase in the concentration of the HCl eluent. The elution yield was the lowest (18 %) when the generator was eluted with 0.2 M HCl, and increased to 66 % with 0.4 M HCl, 99 % with 0.6 M HCl, 118 % with 0.8 M HCl and 126 % with 1.0 M HCl.

4.4.3 Determination of ^{68}Ge breakthrough

The ^{68}Ge breakthrough was the lowest at 0.004 % when eluting the generator with 0.2 M HCl, then remained the same (0.008 %) when eluting with 0.4 M and 0.6 M HCl. There was however a radical increase in the ^{68}Ge breakthrough when using 0.8 M HCl (0.591 %) and 1.0 M (0.62 %) as eluents.

4.4.4 Determination of metal contaminants

The metal contaminants (with the exception of Fe) were consistently lowest when eluting with 0.2 M HCl. The Fe content was fairly consistent with no particular trend and was 0.32 ppm when 0.2 M HCl was used as the eluent, 0.34 ppm for 0.4 M HCl, 0.39 ppm for 0.6 M HCl, 0.23 ppm for 0.8 M HCl and 0.34 ppm for 1.0 M HCl.

The Zn content increased consistently with the increase in HCl concentration of the eluents with the lowest Zn content (0.42 ppm) using 0.2 M HCl as eluent, 1.35 ppm for 0.4 M HCl, 1.55 ppm for 0.6 M HCl, 1.67 ppm for 0.8 M HCl and 2.31 ppm for 1.0 M HCl.

The Ga content remained fairly constant between 0.2 M – 0.6 M HCl concentration of the eluents (0.15 ppm – 0.17 ppm) and then increased consistently to 0.40 ppm for 0.8 M HCl and 0.47 ppm for 1.0 M HCl.

There was no Cu present in any of the ^{68}Ga eluates from the different eluent HCl concentrations except for the 1.0 M HCl (0.03 ppm).

4.5 Discussion

The elution profiles (Figures 4.1 – 4.6) show that the ^{68}Ga activity eluted (generator elution yield) decreased consistently when using lower HCl concentrations as eluents. This result concurs with the study of de Blois et al. (2011), which showed that elution with 0.6 M HCl resulted in 25 % less activity than elution with 1 M HCl. The elution yield results of the current

study show that an HCl concentration of 0.2 M HCl resulted in an extremely low 18 % elution yield, while concentrations of 0.6 M and upwards resulted in average elution yields above 99 % with the maximum of 126 % with 1.0 M. Activities in excess of 100 % were due to the double-loading of ^{68}Ge onto the generator column and the expression of eluted activity as a percentage of the nominal ^{68}Ge activity. The study by Loc'h et al. (1980) found the elution yield to be 78 – 80 %, using 1 M HCl as eluent, while 75 % of the generator activity was eluted in 2.5 ml eluate. Aardaneh and Van der Walt (2006) found that 3 ml 1 M HCl eluted more than 95 % of available activity (65 % of total ^{68}Ga on the double-loaded column). Das et al. (2013) found the elution yield to be 65 % after initially loading the ^{68}Ge activity but dropped to about 55 % after 200 days. Rossouw and Breeman (2012) found that 95 % of the total ^{68}Ga activity eluted in the first 4 ml 0.6 M fraction. Due to the different calculation methods used, the elution yield data given for some of the other studies cannot be directly compared with those obtained in this study, in which the iThemba LABS generator was used, and are therefore only given for the record.

In this study, the ^{68}Ge breakthrough increased consistently with increase in HCl concentration as an eluent. The ^{68}Ge breakthrough was lowest when eluting the generator with 0.2 M HCl (0.004 %) and 0.008 % when using both 0.4 M and 0.6 M as eluents. However, the ^{68}Ge breakthrough increased dramatically to 0.591 % and 0.620 % when eluting with 0.8 M and 1.0 M HCl respectively. This result is in contrast with that of Das et al. (2013), in which the ^{68}Ge breakthrough was 0.023 % after 140 elutions, using 1 M HCl as eluent. These contrasting results could probably be attributed to differences in the generator column matrix preparation. Loc'h et al. (1980) also found that the stronger acidity of the eluent resulted in greater ^{68}Ga activity eluted but also a greater ^{68}Ge contamination (the ^{68}Ge contamination was 10^{-4} % per elution in 1.0 M HCl). In the study by Aardaneh and Van der Walt (2006), the breakthrough of ^{68}Ge was found to be 6.1 x 10^{-4} % (in 1.0 M HCl). De Blois et al. (2011) found that the ^{68}Ge activity in the eluates remained constant when eluting the generator with lower concentration eluents.

The eluates from the different eluents (0.2 M to 1.0 M) contained only small quantities (< 3.2 ppm) of the total quantity of metal ions tested and increased consistently with increasing HCl molarity. The concentration of the total metals was lowest in the 0.2 M HCl eluate and increased consistently with increase in HCl concentration of the eluent. Of all the metals tested, Zn is the only one to increase consistently with increasing molarity of HCl, and is therefore by far the main contributor to the increase of total metals with increase in HCl

concentration of the eluent. The relatively low levels of Fe and, especially Cu, obtained in this study, are very important considering the findings by Oehlke et al. (2013). According to their findings, Cu^{2+} and Fe^{3+} are the metal cations with the strongest competitive behaviour for Ga^{3+} in the radiolabelling of DOTA-peptides. When ^{68}Ga eluates are not pre-purified, high levels of Cu^{2+} and Fe^{3+} could lead to lower labelling yields. High Zn^{2+} levels have a much lower effect in this respect. Loc'h et al. (1980) also tested for metal contaminants (Sn, Ba, Co, Cr, Fe, Hg, Sb and Zn) in 5 ml 1 M HCl eluate. The highest metal contamination was from Sn (0.5 µg/ml), then Fe (0.18 µg/ml) and Zn (0.16 µg/ml). In the study by de Blois et al. (2011), concentrations of Ga, Ge, Zn, Ti, Sn, Fe, Al and Cu were determined only for the 0.6 M HCl eluates. They found that metal ions in the eluate were always < 10 ppm and for Zn < 3 ppm and for all the other metals < 1 ppm. In the study by Das et al. (2013), Sn was the only metal tested for and found to be 0.03 ppm. Rossouw and Breeman (2012) tested for metal ion content (Zn, Fe, Sn, Ti, Cu, Al) using 0.6 M HCl and found the following content in a 1 month old generator: Zn (1.33 ppm), Fe (0.24 ppm), Sn (0.24 ppm), Ti (0.08 ppm), Cu (0.02 ppm) and Al (0.72 ppm). They found that the metal content did not change significantly over a period of 1 year with no significant increase of Sn in eluates over time (SnO_2 does not leak despite continuous elution with 0.6 M HCl).

The European Pharmacopoeia (2013) specification states that no more than 0.001 % of ^{68}Ge should be present in the ^{68}Ga eluate for radiolabelling. ^{68}Ge breakthrough level specifications of all producers is less than 0.01 %, but it may be higher, particularly when the generator is not eluted daily (de Blois et al., 2011). Metal and ^{68}Ge contamination can be reduced by regular elution, elution prior to the synthesis and purification of the eluate or labelled product (Velikyan, 2015). Zn ions are present in the eluate because ^{68}Ga decays to ^{68}Zn.

4.6 Conclusion

An increase in the HCl concentration of the eluent resulted in increasing generator elution yields but also higher ^{68}Ge breakthrough, especially with HCl concentrations in excess of 0.6 M. The latter finding is in contrast with those of a few other investigators. Zn is the only metal tested for that shows a consistent increase with increasing HCl concentrations. This could affect labelling if eluents contain very high concentrations of HCl and eluates are not pre-purified, even taking into account that Zn^{2+} has a lower effect on labelling than metals such as Fe^{3+} and Cu^{2+}. The increased ^{68}Ge breakthrough, resulting from the use of eluents containing high concentrations of HCl, could be problematic with regards to radiation safety and radioactive waste control. Even if ^{68}Ge can be efficiently excluded from

radiopharmaceutical products by means of pre-labelling purification of eluates and/or post-labelling purification of labelled products, the proper disposal of ^{68}Ge-containing waste should still be dealt with. In order to compromise optimal elution yield with minimal ^{68}Ge breakthrough, based on the results of this study, 0.6 M HCl should therefore be the eluent of choice for a SnO_2-based generator.

Chapter 5: Optimization of radiolabelling parameters using the fractionated elution method

5.1 Introduction

The SnO_2-based generator, as well as radiolabelling conditions, using the 0.6 M HCl containing eluates, was well described by de Blois et al. (2011), Rossouw and Breeman (2012) and Sudbrock et al. (2014), but additional investigation into aspects of radiolabelling with ^{68}Ga from this generator is required.

De Blois et al. (2011) performed DOTATATE-labelling studies with ^{68}Ga eluted from a SnO_2-based generator, but with maximum reaction volumes of 1.5 ml. Their study was only performed with DOTATATE and its main focus was to investigate the characteristics of the SnO_2 generator and eluate purification with less focus on radiolabelling. Rossouw and Breeman (2012) investigated a method to prepare [^{68}Ga]DOTATATE using fractionated, un-purified ^{68}Ga eluates by maximising the eluted activity for the labelling and adapting existing labelling methods to accommodate for the large volume and more acidic eluate. Their study also describes a rough optimization of the DOTATATE content of labelling mixtures to ensure optimal radiolabelling yields by employing two peptide masses (30 and 50 µg). In this same study labelling efficiencies were compared after 2 – 3 days of non-elution of the generator. The study by Sudbrock, et al. (2014) on [^{68}Ga]DOTATATE, investigated how factors such as the quantity of DOTATATE, addition of Fe^{3+} salts and the use of sodium acetate buffer (instead of HEPES) influence the radiochemical synthesis. Their study was only performed with DOTATATE and with peptide masses of 1 – 15 µg in a reaction volume of approximately 2 ml.

In this investigation, some of the gaps left by the aforementioned investigations were addressed, as outlined in section 2.2.2. For this purpose, the three important DOTA-conjugated somatostatin analogues DOTATATE, DOTATOC and DOTANOC were all selected to serve as labelling substrates. The study has the additional value of the labelling and subsequent processing of all three of these DOTA-peptides being described together.

5.2 Aim

The main purpose of this investigation was to determine the influence of various quantities (15 – 50 µg) of three DOTA-conjugated peptide ligands (DOTATATE, DOTATOC and DOTANOC) on labelling efficiencies and radiochemical yields when labelled with fractionated ^{68}Ga eluates obtained from a SnO_2-based ^{68}Ge/^{68}Ga generator. Further aims were

to investigate the influence of extended heating times (25 minutes versus 15 minutes), as well as the heating capabilities of two different heating modes (water-bath versus a heating block) on labelling efficiency and yield. A final aim was to determine the influence of various periods of non-elution of the $^{68}Ge/^{68}Ga$ generator on labelling efficiencies, RC yields and metal ion content of the ^{68}Ga eluates.

5.3 Materials and methods

General methods are summarised in chapter 3. This section describes variations and details specific to the work described in this chapter.

5.3.1 Elution of the generator

^{68}Ga was eluted from the 1850 MBq (50 mCi) SnO_2-based $^{68}Ge/^{68}Ga$ generator with 0.6 M HCl (see section 3.4.1). The generator was eluted daily on weekdays, except when the experiments were performed to assess the effects of various periods (3, 7, 14 and 21 days) of non-elution of the generator on the radiolabelling results. For labelling purposes, a fractionated elution method was used, and fractions were collected in 10 ml borosilicate glass vials.

Elution was conducted in two ways. In method 1, a fraction of 1.5 ml was firstly eluted in a pre-elution step, followed by a second fraction of 6.0 ml and finally a third fraction of approximately 2.5 ml in a post-elution step. The first and third fractions were not used for radiolabelling. The second fraction of 6.0 ml was divided into three 2.0 ml sub-fractions with activities ranging from 111 MBq to 630 MBq. These 2 ml fractions were used for the radiolabelling of the three peptide ligands immediately after elution.

In method 2, a fraction of 1.5 ml was firstly eluted in a pre-elution step, followed by a second single fraction of 2.0 ml (with activities ranging from 1006 MBq to 1169 MBq), and finally a third fraction of approximately 6.5 ml in a post-elution step. The second 2 ml fraction was used for high activity labelling of only one peptide ligand at a time.

5.3.2 Radiolabelling of the three DOTA-peptide ligands

The 1 mg/ml DOTA-peptide (DOTATATE, DOTATOC and DOTANOC) stock solutions were prepared using ultra-pure pharmaceutical grade water and stored at -20 °C (see 3.3.2).

For most experiments, a 2 ml portion of the 6 ml second fraction of the eluate was mixed by a vortex action with 575 µl of 2.5 M sodium acetate solution in a glass vial to render a buffered mixture at a suitable pH. Various quantities of the three peptide ligands (15, 25, 35 and 50 µg) were introduced to the buffered ^{68}Ga activity from their respective stock solutions, the vials were sealed and labelling mixtures were heated in a heating block for 15 min at 95 °C. Experiments with heating times of 25 min, using 35 µg DOTA-peptide, were also conducted, as well as experiments using a water-bath set at 95 °C instead of a heating block. The higher activity 2 ml fraction eluted from the generator was buffered in a similar way and radiolabelling was conducted using only 35 µg of DOTA-peptide, followed by heating in a heating block for 15 min at 95 °C. Radiolabelling experiments were also performed using ^{68}Ga eluted after various periods of non-elution of the ^{68}Ge/^{68}Ga generator (3, 7, 14 and 21 days). The eluate fractionation and sub-division of the second fraction was carried out similarly to method 1 described above.

5.3.3 Sep-Pak C18 post-radiolabelling purification

After the heating process, a sample with a maximum volume of 50 µl (depending on the radioactivity concentration) was taken from the radiolabelling reaction solution for HPLC analysis (see 5.3.4 below). The radiolabelled mixtures were then purified on a 500 mg, 3 cc, Sep-Pak C18 cartridge according to the method described in 3.4.2. Sep-Pak purification conditions for ^{68}Ga-labelled DOTANOC were established as follows: Radiolabelling mixtures (in which 35 µg DOTANOC had been used) were loaded on a pre-conditioned Sep-Pak C18 cartridge according to the procedure described in 3.4.2. Ethanol:saline mixtures of either 50:50 or 70:30 were used to collect the labelled DOTATATE in the **S2** fraction.

5.3.4 Quality Control

Radiolabelling efficiency or labelling efficiency was obtained from the HPLC analysis of the unpurified labelled product as described in chapter 3. Radiochemical purities of purified ^{68}Ga-labelled DOTA-peptides were determined by means of HPLC.

For radiochemical yield, refer to 3.4.2. The activities in the collected fractions (**S1** and **S2**), as well as the remaining activities in the reaction vial and on the Sep-Pak C18 cartridge were measured in a dose calibrator.

The Fe, Zn, Ga, Sn and Cu concentrations were determined in the ^{68}Ga eluates after various periods of non-elution of the generator. Standard solutions (1 ppm, 5 ppm and 10 ppm) of Fe, Zn, Ga, Sn and Cu were prepared as described in section 3.3.5.

5.4 Results

5.4.1 Elution of the generator

At the end of 12 months, a total activity of 555 MBq could still be eluted from the generator. In method 1 of the elution of the generator, activities in the 2 ml sub-fractions of the eluate used for the radiolabelling experiments ranged from 111 MBq to 630 MBq per sub-fraction, depending on the age of the generator. This represented a total of approximately 333 MBq to 1890 MBq activity for the whole 6 ml fraction. The 6 ml fraction comprised of approximately 95 % of the total activity per elution with each 2 ml sub-fraction further representing about 32 % thereof. In method 2, activities in the 2 ml main fraction ranged from 1006 MBq to 1169 MBq. The 2 ml fraction represented a concentrated activity of about 87 % of the total activity per elution.

5.4.2 Radiolabelling results

5.4.2.1 Sep-Pak C18 cartridge conditions for the purification of ^{68}Ga-labelled DOTANOC

Purification data for [^{68}Ga]DOTANOC using ethanol:saline 50:50 and 70:30 as eluent, are presented in Table 5.1. When 50 % ethanol was used as eluent, the eluted activity of the labelled product in the **S2** fractions (radiochemical yield) was much lower than the **S2** activity obtained when 70 % ethanol eluent was used as the eluent (37 – 41 % compared to 75 – 84 %). The remaining activity on the Sep Pak C18 cartridge (**S4**) for the ethanol:saline 50:50 was about four to five times more than when ethanol:saline 70:30 was used as the eluent (35 – 37 % compared to 7 – 9 %).

Table 5.1: Comparison of Sep-Pak C18 purification data of [^{68}Ga]DOTANOC, obtained using eluents with different ethanol:saline volume ratios

Sep-Pak C18 purification data of [^{68}Ga]DOTANOC, using fractionated ^{68}Ga eluates showing single values of decay-corrected activities in various fractions, expressed as percentages of starting activities. Radiolabelling conditions: 35 µg DOTANOC in reaction volume 2.61 ml; reaction temperature = 95 ˚C; reaction time = 15 min.; S1 = first Sep-Pak fraction collected with 8 ml saline; S2 (Radiochemical Yield) = second Sep-Pak fraction collected with 0.7 ml ethanol:saline [50:50 or 70:30] + 2.25 ml saline; S3 = remaining activity in vial; and S4 = remaining activity in Sep-Pak C18 cartridge after collecting S2

EtOH:saline ratio (v/v)	**S1 (%)**	**S2 (%)**	**S3 (%)**	**S4 (%)**
50:50	10	41	16	35
	13	37	14	35
	7	41	14	37
70:30	3	81	5	9
	3	84	4	7
	6	75	8	9

5.4.2.2 Peptide ligand content

Radiolabelling efficiency and Sep-Pak C18 purification data of the three ^{68}Ga-labelled DOTA-peptides, using fractionated ^{68}Ga eluates from a ^{68}Ge/^{68}Ga generator are presented in Table 5.2. The results present data on radiochemical yields (second fraction, **S2**) as a function of the molar concentration of DOTATATE, DOTATOC and DOTANOC used. Percentage activities present in the first washout fraction (**S1**), as well as retained activity in the reaction vial (**S3**) and on the C18 cartridge post desorption (**S4**) are also given.

Table 5.2: Radiolabelling efficiency and Sep-Pak C18 purification data of ^{68}Ga-labelled DOTA-peptides, using fractionated ^{68}Ga eluates from a ^{68}Ge/^{68}Ga generator

Radiolabelling efficiency and Sep-Pak C18 purification data of ^{68}Ga-labelled DOTA-peptides, using fractionated ^{68}Ga eluates from a ^{68}Ge/^{68}Ga generator, showing mean values of decay-corrected activities in various fractions, expressed as percentages of the starting activity (111 – 630 MBq or 1006 – 1169 MBq*). Radiolabelling conditions: 15 – 50 µg DOTA-peptide in reaction volume 2.59 – 2.63 ml; reaction temperature = 95 ˚C; reaction time = 15 min.; n = number of experiments; S1 = first Sep-Pak fraction collected with 8 ml saline; S2 (Radiochemical Yield) = second Sep-Pak fraction collected with 0.7 ml ethanol:saline [50:50 or 70:30 for DOTANOC] + 2.25 ml saline; S3 = remaining activity in vial; S4 = remaining activity in Sep-Pak C18 cartridge after collecting S2

Peptide	Peptide mass (µg)	Peptide molar conc. (µM)	Labelling efficiency (%)	S1 (%)	S2 (%)	S3 (%)	S4 (%)
DOTATATE	15 (n=5)	4.0	84 ± 13	11 ± 10	55± 16	22 ± 9	9 ± 1
	25 (n=6)	6.7	94 ± 5	5 ± 2	71 ± 4	17 ± 4	6 ± 3
	35 (n=6)	9.3	97 ± 1	3 ± 1	72 ± 5	16 ± 4	8 ± 3
	35* (n=3)	9.3	98 ± 1	3 ± 1	75± 3	13 ± 2	8 ± 4
	50 (n=4)	13.3	98 ± 1	3 ± 1	68 ± 4	20 ± 5	8 ± 2
DOTATOC	15 (n=5)	4.1	80 ± 19	15 ± 15	56 ± 20	22 ± 8	6 ± 2
	25 (n=6)	6.8	97 ± 2	4 ± 2	71 ± 4	17 ± 4	7 ± 2
	35 (n=6)	9.4	97 ± 1	3 ± 2	75 ± 7	11 ± 4	9 ± 4
	35* (n=3)	9.4	99 ± 1	3 ± 1	73 ± 3	14 ± 3	10 ± 1
	50 (n=4)	13.4	98 ± 1	2 ± 1	77 ± 3	13 ± 2	7 ± 2
DOTANOC	15 (n=5)	4.0	56 ± 30	26 ± 23	34 ± 27	29 ± 11	9 ± 2
	25 (n=3)	6.6	97 ± 1	3 ± 1	69 ± 5	17 ± 4	6 ± 4
	35 (n=5)	9.2	96 ± 1	3 ± 2	76 ± 4	11 ± 4	6 ± 2
	35* (n=3)	9.2	100 ± 1	2 ± 0	71± 6	15 ± 2	11 ± 5
	50 (n=4)	13.1	98 ± 2	3 ± 2	74 ± 3	17 ± 2	7 ± 1

Statistically, there was a significant impact by the various peptide masses/molar concentrations on the labelling efficiencies of the three DOTA-peptides ($p = 0.02$) (see Figure 5.1), particularly in case of the 4 µM DOTA-peptide concentration (15 µg in 2.59 ml). Labelling efficiencies for the three DOTA-peptides were all similar for all peptide masses, except for DOTANOC when 15 µg was used. The reason for this miss-match result is not clear. The use of 4 µM (15 µg) DOTA-peptide resulted in the lowest radiolabelling efficiency,

an average of 84 % with DOTATATE, 80 % with DOTATOC and 56 % with DOTANOC. This also resulted in the lowest and most inconsistent radiochemical yields (55 % for DOTATATE, 56 % for DOTATOC, 34 % for DOTANOC) and the highest retained activity on the glass vial with an average of 22 % for both DOTATATE and DOTATOC and 29 % for DOTANOC. A DOTA-peptide molar concentration of 6.6 – 6.8 µM (25 µg in 2.60 ml) resulted in labelling efficiencies of 94 – 97 %, with concurring radiochemical yields of 69 – 71 %. A DOTA-peptide molar concentration of 9.2 – 9.4 µM (35 µg in 2.61 ml) resulted in labelling efficiencies consistently above 95 %, with concurring radiochemical yields of 72 – 76 %. A similar result was obtained when using a higher activity ^{68}Ga eluate fraction (elution method 2), except for DOTANOC where a slightly lower yield of 71 % was obtained. A DOTA-peptide molar concentration of 13.1 – 13.4 µM (50 µg in 2.625 ml) consistently resulted in labelling efficiencies of 98 % with concurring radiochemical yields of 68 – 77 %. The difference in the radiochemical yields obtained from the different peptide masses/molar concentration was found to be statistically significant ($p = 0.000$) particularly with 15 µg (4 µM). The mass/molar concentration of 35 µg (9.2 – 9.4 µM) resulted in the highest radiochemical yield (see Figure 5.2). Combined data were presented in Figure 5.2 because there was no statistically significant difference between the three DOTA-peptides for the impact of various peptide masses/molar concentrations on the radiochemical yields.

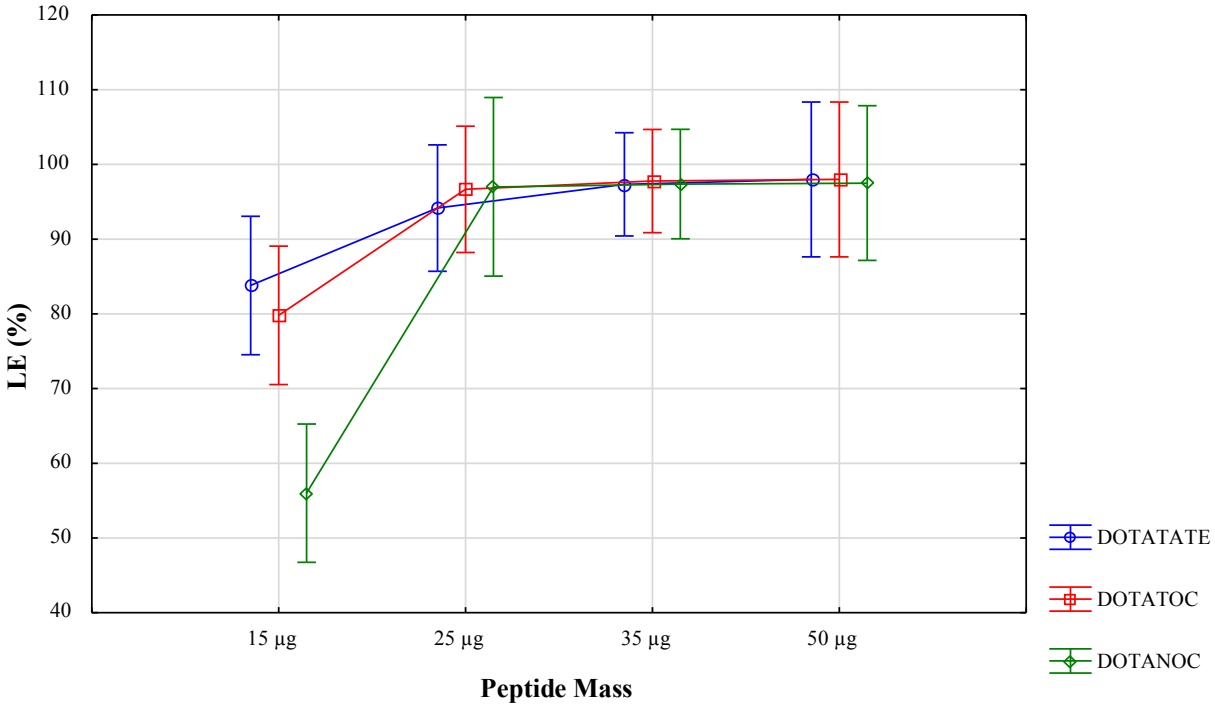


Figure 5.1: Radiolabelling efficiency profile for various masses of three DOTA-peptides

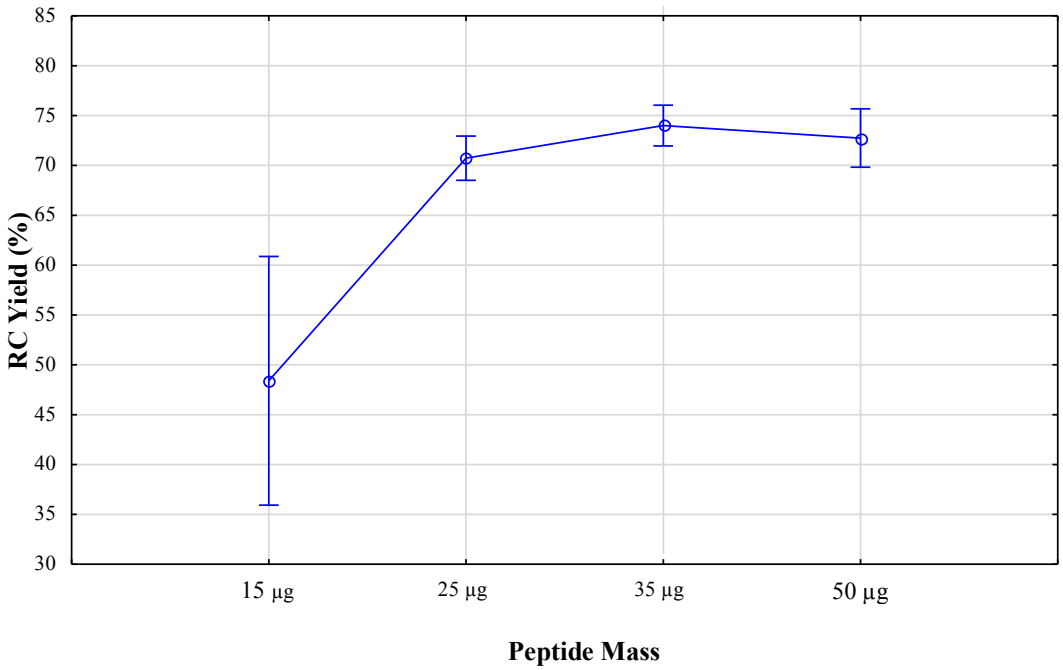


Figure 5.2: Radiochemical yield profile for various masses of three DOTA-peptides, providing combined data

5.4.2.3 Reaction heating time and comparison of heating methods

The influence of extended reaction (heating) times (25 min versus 15 min) on labelling yields, radiochemical yields, and other parameters, is displayed in Table 5.3. The average labelling efficiency for DOTATATE was the same (97 %) for both 15 and 25 min, for DOTATOC it was slightly higher for 15 min (97 % compared to 96 %) and only for DOTANOC it was slightly higher for 25 min (98 % versus 96 %). The radiochemical yield (**S2**) however, was consistently marginally higher for all three DOTA-peptides for the 25 min heating time ($p = 0.006$) (see Figure 5.3).

Table 5.3: Influence of two different reaction times on radiolabelling results of ^{68}Ga-labelled DOTA-peptides

Radioabelling efficiency and Sep-Pak C18 purification data after labelling fractionated ^{68}Ga eluates and 35 µg DOTA-peptide, comparing 25 min versus 15 min heating time, showing mean values of decay-corrected activities in various fractions, expressed as percentages of the starting activity, number of experiments given within brackets; S1 = first Sep-Pak fraction collected with 8 ml saline; S2 (Radiochemical Yield) = second Sep-Pak fraction collected with 0.7 ml ethanol:saline [50:50 or 70:30 for DOTANOC] + 2.25 ml saline; S3 = remaining activity in vial; and S4 = remaining activity in Sep-Pak C18 cartridge after collecting S2

Peptide	Reaction time (min)	*Labelling efficiency (%)	S1 (%)	S2 (%)	S3 (%)	S4 (%)
DOTATATE	15 (n=5)	97 ± 1	3 ± 1	71 ± 4	16 ± 3	8 ± 3
	25 (n=4)	97 ± 2	2 ± 1	75 ± 6	15 ± 1	8 ± 4
DOTATOC	15 (n=5)	97 ± 1	3 ± 2	72 ± 4	12 ± 4	9 ± 4
	25 (n=5)	96 ± 1	3 ± 1	77 ± 3	12 ± 3	7 ± 2
DOTANOC	15 (n=5)	96 ± 1	3 ± 2	76 ± 4	11 ± 4	6 ± 2
	25 (n=5)	98 ± 1	2 ± 1	82 ± 5	9 ± 6	6 ± 2

*Determined by means of HPLC

Radiolabelling results are compared for different heating methods (water-bath and heating block) in Table 5.4 and show no significant difference between the two methods. For two of the DOTA-peptides (DOTATATE and DOTANOC), the radiolabelling efficiency was slightly higher using the water-bath compared to the heating block. The average radiochemical yield (**S2**) for DOTATATE was also slightly higher for the water-bath experiments (75 % compared to 71 %), slightly higher for DOTATOC (73 % compared to 72 %) and the same for DOTANOC (76 %). The remaining activity on the vial for the heating block experiments was slightly lower for DOTATOC and DOTANOC (13 % compared to 11 % for both DOTA-peptides) and slightly higher for DOTATATE (16 % compared to 12 %).

Table 5.4: Influence of two different heating methods on radiolabelling results of ^{68}Ga-labelled DOTA-peptides

Radiolabelling efficiency and Sep-Pak C18 purification data of ^{68}Ga-labelled DOTA-peptides, using fractionated ^{68}Ga eluates and 35 µg DOTA-peptide, comparing 15 min heating time (reaction time) in a water-bath versus 15 min heating time (reaction time) in a controlled heating block, showing mean values of decay-corrected activities in various fractions, expressed as percentages of the starting activity, number of experiments given within brackets; S1 = first Sep-Pak fraction collected with 8 ml saline; S2 (Radiochemical Yield) = second Sep-Pak fraction collected with 0.7 ml ethanol:saline [50:50 or 70:30 for DOTANOC] + 2.25 ml saline; S3 = remaining activity in vial; and S4 = remaining activity in Sep-Pak C18 cartridge after collecting S2

Peptide	Heating method (15min)	*Labelling efficiency (%)	S1 (%)	S2 (%)	S3 (%)	S4 (%)
DOTATATE	Water-bath (n=3)	99 ± 1	3 ± 1	75 ± 2	12 ± 3	10 ± 3
	Heating block (n=5)	97 ± 1	3 ± 1	71 ± 4	16 ± 3	8 ± 3
DOTATOC	Water-bath (n=3)	95 ± 5	7 ± 4	73 ± 6	13 ± 3	6 ± 1
	Heating block (n=5)	97 ± 1	3 ± 2	72 ± 4	12 ± 4	9 ± 4
DOTANOC	Water-bath (n=3)	99 ± 2	3 ± 3	76 ± 1	13 ± 1	5 ± 3
	Heating block (n=5)	96 ± 1	3 ± 2	76 ± 4	11 ± 4	6 ± 2

*Determined by means of HPLC

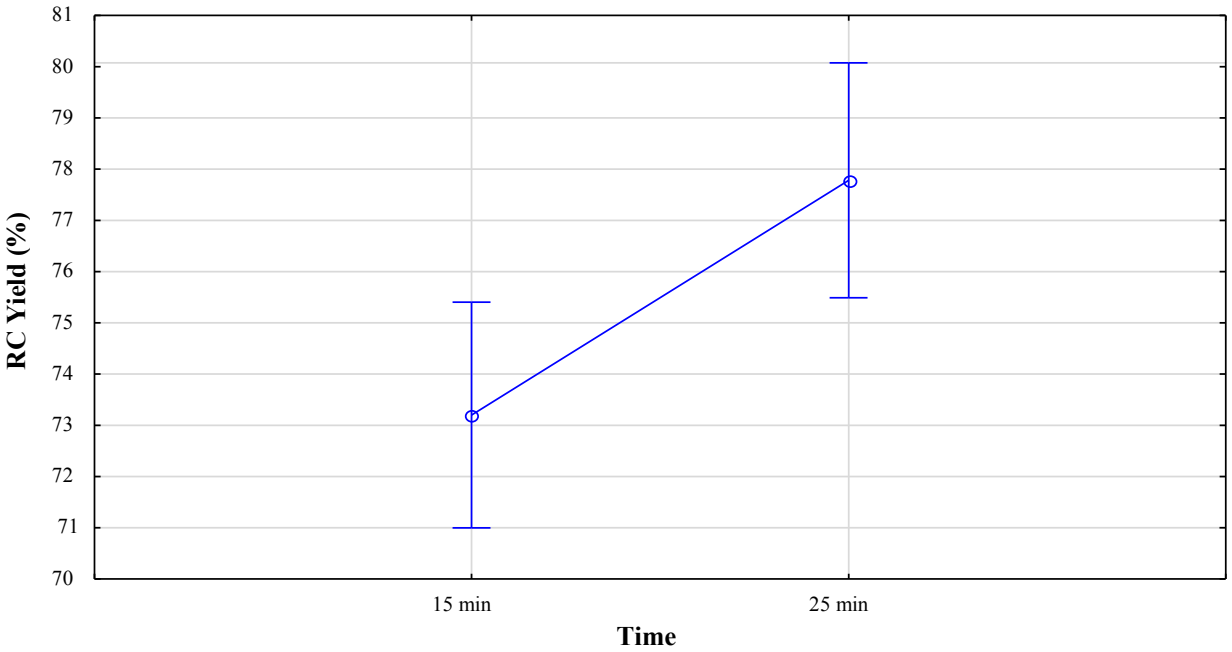


Figure 5.3: Radiochemical yield profile for 35 µg DOTA-peptide, using different heating times, providing combined data

5.4.2.4 The influence of non-elution of the $^{68}Ge/^{68}Ga$ generator

Radiolabelling efficiency and Sep-Pak C18 purification data of ^{68}Ga-labelled DOTA-peptides, using fractionated ^{68}Ga eluates obtained after various periods (3, 7, 14 and 21 days) of non-elution of the $^{68}Ge/^{68}Ga$ generator are displayed in Table 5.5.

The combined data for the three DOTA-peptides show that there was no major decline in the radiolabelling efficiency until 7 days of non-elution, but during the period between 7 and 14 days there appears to be the most significant decline. Statistically, there was found to be a significant difference between the radiolabelling efficiencies of the three DOTA-peptides ($p = 0.03$) after the various periods of non-elution of the generator (see Figure 5.4).

If all three peptides are considered together, there was a statistically significant difference in the radiochemical yield over the periods of non-elution of the generator ($p = 0.000$) (see Figure 5.5). Between the 0-day and 14-day period there was a steady decline in radiochemical yield (74 % to 63 %), but there was no further decline between 14 and 21 days of non-elution. The biggest drop in radiochemical yield was apparent between 7 and 14 days of non-elution. There was no significant difference between the radiochemical yields of the individual three DOTA-peptides over the periods of non-elution ($p = 0.11$) (see Figure 5.6). From the 0 until the 21-day period there was no major increase in free (un-complexed) ^{68}Ga as shown by the **S1** values in Table 5.5. Activity retained in reaction vials also appears to remain constant, but there was a steady increase in the remaining activity in the Sep-Pak C18 cartridge, especially between the 7 and 14-day period. The total metal ion content (11.1 ppm) (see Figure 5.7) as well as the Fe and Zn content (1.6 and 5.0 ppm respectively) of the ^{68}Ga eluates was particularly high during this period of non-elution (see results in Table 5.6).

Table 5.5: Radiolabelling results of ^{68}Ga-labelled DOTA-peptides, using fractionated ^{68}Ga eluates obtained after various periods of non-elution of the ^{68}Ge/^{68}Ga generator

Radiolabelling efficiency and Sep-Pak C18 purification data of ^{68}Ga-labelled DOTA-peptides, using fractionated ^{68}Ga eluates obtained after various periods of non-elution of the ^{68}Ge/^{68}Ga generator, showing mean values of decay-corrected activities in various fractions, expressed as percentages of the starting activity and number of experiments given within brackets. Radiolabelling conditions: 35 µg DOTA-peptide in reaction volume 2.61 ml; reaction temperature = 95 ˚C; reaction time = 15 min.; n = number of experiments; S1 = first Sep-Pak fraction collected with 8 ml saline; S2 (Radiochemical Yield) = second Sep-Pak fraction collected with 0.7 ml ethanol:saline [50:50 or 70:30 for DOTANOC] + 2.25 ml saline; S3 = remaining activity in vial; and S4 = remaining activity in Sep-Pak C18 cartridge after collecting S2

Peptide	No. of days of non-elution	*Labelling effic. (%)	S1 (%)	S2 (%)	S3 (%)	S4 (%)
DOTATATE	0 (n=6)	97 ± 1	3 ± 1	72 ± 5	16 ± 4	8 ± 3
	3 (n=5)	98 ± 1	2 ± 2	73 ± 9	12 ± 7	11 ± 4
	7 (n=5)	98 ± 1	3 ± 1	70 ± 4	16 ± 4	11 ± 4
	14 (n=4)	91 ± 10	5 ± 5	59 ± 2	13 ± 4	20 ± 7
	21 (n=5)	91 ± 6	7 ± 4	60 ± 12	14 ± 3	15 ± 5
DOTATOC	0 (n=6)	97 ± 1	3 ± 2	75 ± 7	11 ± 4	9 ± 4
	3 (n=5)	97 ± 2	2 ± 2	73 ± 9	12 ± 7	11 ± 4
	7 (n=6)	96 ± 3	4 ± 3	71 ± 6	14 ± 4	10 ± 3
	14 (n=5)	96 ± 2	3 ± 2	67 ± 11	11 ± 4	16 ± 11
	21 (n=4)	95 ± 4	4 ± 4	65 ± 14	12 ± 3	16 ± 8
DOTANOC	0 (n=5)	96 ± 1	3 ± 2	76 ± 4	11 ± 4	6 ± 2
	3 (n=5)	98 ± 2	4 ± 2	71 ± 4	13 ± 3	10 ± 3
	7 (n=6)	95 ± 3	5 ± 2	67 ± 11	15 ± 4	14 ± 10
	14 (n=4)	94 ± 7	4 ± 3	61 ± 5	12 ± 2	21 ± 10
	21 (n=3)	92 ± 12	3 ± 2	63 ± 15	13 ± 7	19 ± 7
Combined data for DOTATATE, DOTATOC and DOTANOC	0 (n=17)	97 ± 1	3 ± 2	74 ± 6	13 ± 4	8 ± 3
	3 (n=15)	97 ± 2	3 ± 2	72 ± 7	12 ± 6	11 ± 3
	7 (n=17)	96 ± 3	4 ± 2	69 ± 8	15 ± 4	11 ± 6
	14 (n=13)	94 ± 7	4 ± 3	63 ± 8	12 ± 3	19 ± 9
	21 (n=12)	93 ± 7	5 ± 4	63 ± 12	13 ± 4	16 ± 6

*Determined by means of HPLC

Table 5.6: Metal ion content of ^{68}Ga eluates obtained after various periods of non-elution of the $^{68}Ge/^{68}Ga$ generator

Period of non-elution (days)	Labelling efficiency (%)	Al (ppm)	Fe (ppm)	Sn (ppm)	Zn (ppm)	Cu (ppm)	Total Metals (ppm)
3	94	2.0	0.3	1.8	5.7	0.2	10.0
7	94	2.6	0.6	1.1	1.7	0.1	5.9
14	79	2.2	1.6	2.2	5.0	0.4	11.1
21	94	4.2	0.7	1.6	3.4	0.4	10.1

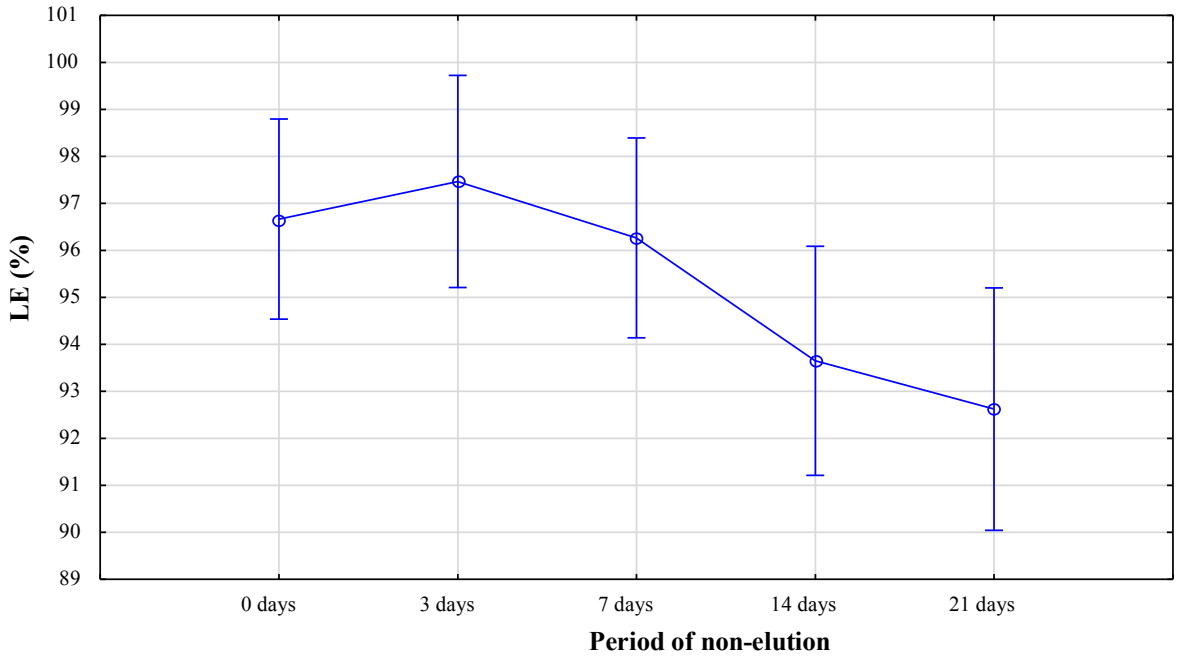


Figure 5.4: Radiolabelling efficiency profile for 35 µg DOTA-peptide for different periods of non-elution of the generator, providing combined data

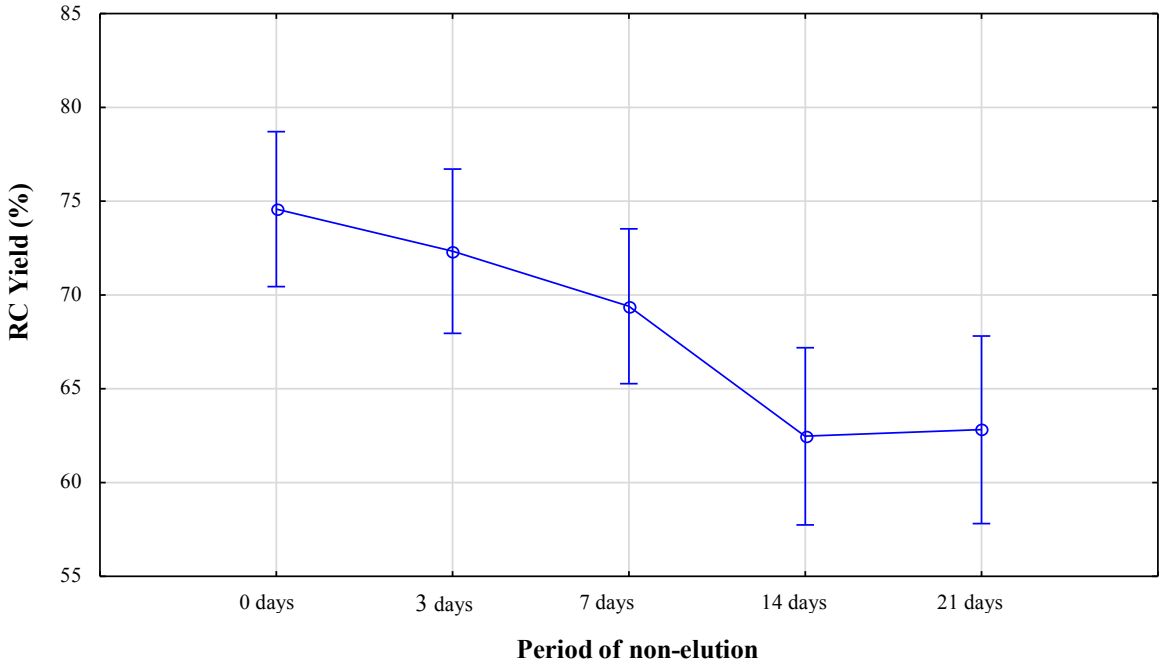


Figure 5.5: Radiochemical yield profile for 35 µg DOTA-peptide for different periods of non-elution of the generator, providing combined data

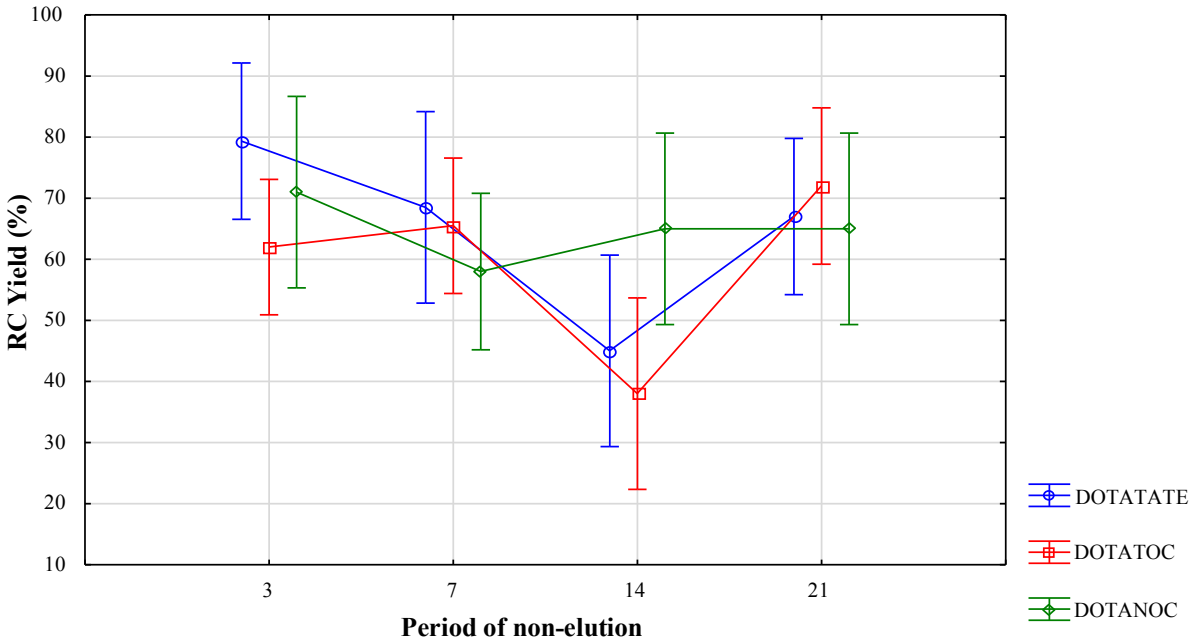


Figure 5.6: Radiochemical yield profile for three DOTA-peptides (35 µg) for different periods of non-elution of the generator

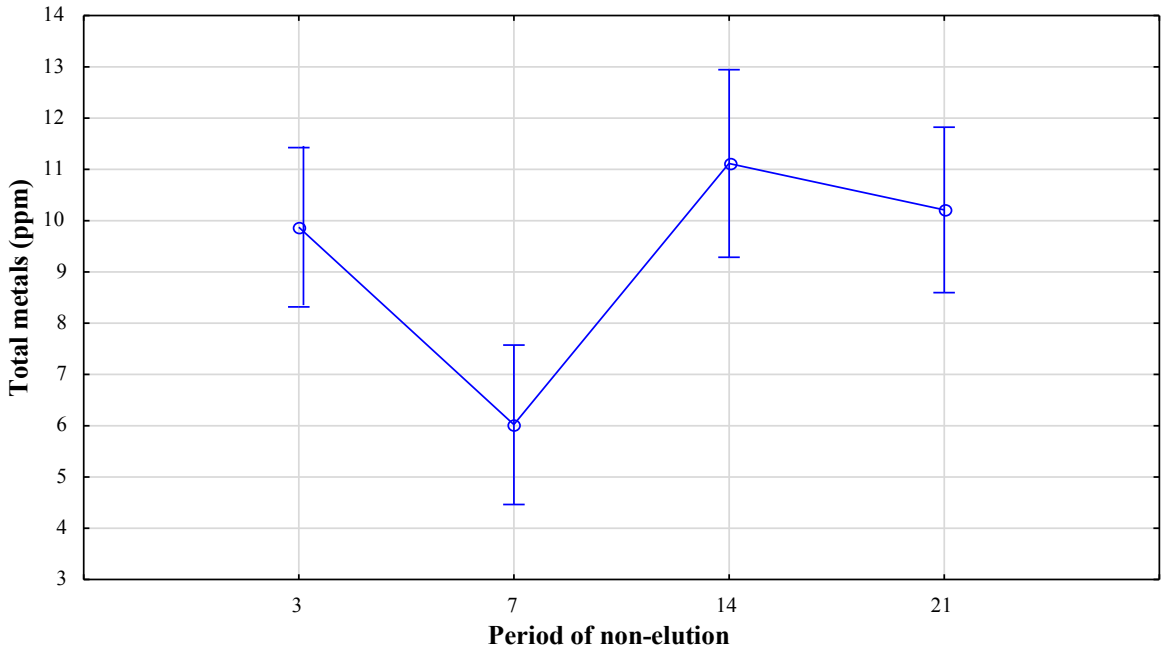


Figure 5.7: Total metal contaminant profile of ^{68}Ga eluates for different periods of non-elution of the generator

5.5 Discussion

Eluate fractionation and sub-division of fractions were employed as a means to introduce ^{68}Ga to labelling mixtures. Labelling and post-labelling purification methods were largely based on the work done by Rossouw and Breeman (2012), but post-labelling purification procedures for radiolabelled DOTANOC was further adapted due to the more lipophilic nature of this peptide molecule (Wild, et al. 2003, 2013). The scales of the labelling and the purification parameters were slightly downsized in order to facilitate the labelling of all three DOTA-peptides in a single elution. This study involved a more comprehensive investigation on the influence of peptide mass/molar concentration on radiolabelling efficiency and radiochemical yields, using all three mentioned DOTA-peptides. Different heating modes of labelling mixtures were implemented and extension of the heating period was performed in order to observe its impact on the labelling parameters of the DOTA-peptides. The influence of the period of non-elution of the generator on radiolabelling efficiencies and radiochemical yields was extended to a maximum period of 21 days. These results were related to the metal analysis of the eluates.

Rossouw and Breeman (2012) found inconsistent labelling efficiencies when using 30 µg of DOTATATE compared to consistently high labelling efficiencies when using 50 µg DOTATATE. These labelling reactions were, however, conducted in bigger volumes ranging

from 3.2 to 5.7 ml and resulted in DOTA-peptide concentrations ranging from 3.9 to 5.5 µM when using 30 µg of DOTATATE and 6.7 to 9 µM when using 50 µg DOTATATE. In this study labelling was conducted in a smaller volume of approximately 2.6 ml. When using 15 µg peptide (4 µM molar concentration), this was found to be too low to result in an acceptable labelling efficiency and radiochemical yield (see **S2**, Table 5.2). The use of 25 µg peptide (6.6 – 6.8 µM molar concentration) resulted in an acceptable but slightly inferior radiolabelling result than 35 µg (9.2 – 9.4 µM). These results corroborate the results of Rossouw and Breeman (2012) and support the assumption that peptide concentration rather than mass should be used as the main influencing parameter on labelling efficiency. It also illustrates the advantage of conducting labelling reactions in small volumes in order to avoid the use of excessive amount of peptide. The use of 13.1 – 13.4 µM (50 µg) shows no dramatic increase in radiolabelling efficiency and radiochemical yield and therefore 35 µg (9.2 – 9.4 µM) appears to be optimum under the conditions of this study. When higher specific activity is required, the use of 6.6 – 6.8 µM (25 µg) peptide could also suffice.

The nature of the remaining activity on the vial (**S3**) is unknown but it is possibly un-reacted ^{68}Ga and not labelled peptide. This is supported by the fact that these **S3** values are generally lower when using higher concentrations of DOTA-peptides, while the **S1** values also drop accordingly. The use of higher peptide concentrations (50 µg, 13 µM), however, could not further reduce **S3** values. The use of alternative types of reactor vials for radiolabelling of peptides might be worthwhile investigating.

The use of 2 ml eluate for the radiolabelling of DOTA-peptides in this study originated from a practical point of view, i.e. to facilitate the simultaneous labelling experiments on three peptides with a single elution (elution method 1). In a real case scenario where probably only one peptide at a time will be labelled under similar conditions, the main eluted fraction volume will be only 2 ml instead of 6 ml (elution method 2). This would mean that a smaller percentage of total eluted activity would be used to label a peptide (approximately 87 %), but the activity (and therefore the concentration) will be higher. A few labelling experiments with higher activity were therefore also performed, using elution method 2 and only one peptide mass of 35 µg. The higher activity labelling results compare favourably with the lower activity (35 µg) results, as shown in Table 5.2.

The HPLC method used in this study was based on gradient elution, whereas the European Pharmacopoeia describes an isocratic method. The rationale for using a gradient elution

method in this work was mainly to obtain a good separation between free ^{68}Ga and labelled peptide, allowing at the same time a relatively fast elution of the latter, and not really to resolve probable impurity peaks. Nevertheless, no significant traces of radiochemical impurities or side-products were detected in HPLC chromatograms of unpurified labelling mixtures, applying the gradient elution method used in this study. Such impurities were not even detected in labelling reactions involving high activity labelling, using 1006 – 1169 MBq starting activities. Other investigators (Mu et al., 2013) detected radiolytic breakdown impurities in ^{68}Ga-labelled DOTATATE with gradient elution when labelling was conducted at high activity around 800 MBq, but a flatter gradient was used as opposed to the one used in this work.

The Sep-Pak C18 purification method for [^{68}Ga]DOTANOC had to be slightly adapted. The results in Table 5.1 show that an eluent ethanol content of 50 % did not suffice for [^{68}Ga]DOTANOC due to the low radiochemical yields (approximately 40 %) with accompanying high remaining activity in the Sep-Pak C18 cartridge **S4** (approximately 36 %). An eluent with an ethanol content of 70 % resulted in acceptable radiochemical yields of about 80%. Further investigation of eluents containing higher percentages of ethanol, was not done in order to avoid excessive levels of ethanol in the purified labelled product.

The slightly superior radiolabelling result of ^{68}Ga-labelled DOTA-peptides for 25 min versus 15 min heating/reaction time is no real advantage due to activity loss to decay. None of the heating modes appear to be superior to the other (see Tables 5.3 and 5.4).

A period of three days of non-elution of the ^{68}Ge/^{68}Ga generator had no dramatic effect on labelling efficiency and radiochemical yield. This is in agreement with the results presented by Rossouw and Breeman (2012). A 7-day non-elution period resulted in a slight drop, while a 14-day period resulted in the biggest drop in radiochemical yield. The increased Fe content after 14 days of non-elution (see Table 5.6) coincides with a drop in radiochemical yields, especially in the case of DOTATATE (Table 5.5). This concurs with the study performed by Oehlke et al. (2013) on the influence of, amongst others, Cu^{2+} and Fe^{3+} on ^{68}Ga-labelling of DOTATATE, which found that the metal ion/ligand ratio plays a critical role on labelling efficiency. There is no clear explanation for the variability in the metal content in eluates over time (Figure 5.7). The decrease in yield and increase in activity retained by the Sep-Pak C18 cartridge appear to go hand in hand. According to Oehlke et al. (2016) higher quantities of Fe^{3+} could lead to the formation of Fe^{3+} colloids that can bind Ga^{3+}. The retained activity on

the Sep-Pak cartridge could therefore most likely be colloidal-bound Ga^{3+}, causing a decrease in radiochemical yield. This theory is further supported by the fact that free (un-complexed) ^{68}Ga washout from the Sep-Pak C18 cartridge remains fairly unchanged during extended periods of non-elution.

5.6 Conclusion

For all three DOTA-peptides**,** a molar concentration of 9.2 – 9.4 µM (35 µg in 2.61 ml) was found to be the optimum to ensure radiolabelling efficiencies consistently greater than 95 % and average radiochemical yields above 70 %. Extended heating periods and the use of various heating modes offer no advantage in terms of obtaining higher radiochemical yields. Results also suggest that radiochemical yields close to 70 % can still be achieved if generators are left non-eluted for up to 7 days. Even after 21 days of non-elution of the $^{68}Ge/^{68}Ga$ generator, average radiolabelling efficiencies were above 90 % and average radiochemical yields in excess of 60 %. The purification of [^{68}Ga]DOTANOC on Sep-Pak C18 cartridges requires only a slight adjustment on the ethanol content of the eluent.

Chapter 6: Development and evaluation of user-friendly single vial DOTA-peptide kit formulations, using the fractionated elution method

The work described in this chapter was published online on 24 March 2017 in Molecular Imaging and Biology Volume 19, pp 817-824 (DOI 10.1007/s11307-017-1077-7).

6.1 Introduction

In order to improve the user-friendliness of the ^{68}Ga labelling process, there is a need to develop stable kit formulations based on optimized labelling methods. This allows instant preparation of ^{68}Ga-labelled DOTA-peptides within a radiopharmacy environment. A kit for radiopharmaceutical preparation is usually contained in a vial and production of the radiopharmaceutical preparation may require additional steps such as heating.

Various reports on the preparation and use of kits for DOTA-peptide labelling have been published. In most of these reports, kit formulations were based on the use of ^{68}Ge/^{68}Ga generators eluted with relatively low concentrations of HCl (0.1 M) (Mukherjee et al., 2014a, 2014c). The stability of the kits was investigated over a 4-month period (Mukherjee et al., 2014a). In another study (Mukherjee et al., 2014b) freeze-dried DOTATATE kits for radiolabelling with both ^{68}Ga (eluted with 0.1 M HCl) and ^{177}Lu were formulated as a theranostic radiopharmaceutical preparation and were evaluated over a period of 6 months. In a study by Das et al. (2014), freeze-dried mixed peptide kits of DOTATATE and DOTANOC were prepared using equal quantities of the two peptides and sodium acetate as a buffer. Kits were freeze-dried and stored at 0 °C. They were used with ^{68}Ga eluted with 0.1 N HCl from a TiO_2 generator and purified on a cation exchange column. Further purification of the ^{68}Ga-labelled mixed peptide was carried out using Sep-Pak C18 cartridges. The stability of the kits during extended storage periods was not reported. A study by Asti et al. (2015) to develop and optimise direct labelling of DOTATOC through a kit-based approach was conducted using eluates from a TiO_2- and a silica-based generator.

Despite the current available literature information on ^{68}Ga-DOTA-peptide kit labelling, more studies are required. These include the composition of kits that are compatible with the use of other types of ^{68}Ge/^{68}Ga generators such as the SnO_2-based generator in which eluents with higher acidity are generally used, different kit manufacturing procedures, evaluation of kit characteristics and stability over extended periods as well as comparison of kit labelling results with those of other labelling procedures. This investigation describes the development

of vacuum-dried single vial DOTA-peptide (DOTATATE, DOTATOC and DOTANOC) kits, formulated with sodium acetate as a buffer and to be used with fractionated 0.6 M HCl eluates from a SnO_2-based $^{68}Ge/^{68}Ga$ generator. In addition, it includes the monitoring of radiochemical yields of the ^{68}Ga-labelled DOTA-peptides obtained after Sep-Pak C18 purification, radiolabelling efficiency and other quality control parameters (pH, sterility and apyrogenicity) over a 12-month period, as well as a comparison of kit labelling results with those of labelling procedures using aqueous DOTA-peptide stock solutions.

6.2 Aim

The aim of this study was to investigate the feasibility of using pre-prepared kit formulations of the three DOTA-peptides in labelling. The development of the single vial DOTA-peptide kit formulations was based on the optimized labelling conditions using the fractionated elution method (see Chapter 5). The primary aim with these kits containing the peptide and buffer was to improve the convenience and user-friendliness of the labelling procedure. The labelling and purification method was based on that in which the peptide stock solutions were used.

A secondary aim was to carry out labelling over a period of 12 months in order to establish how long such frozen kits could be stored before any deterioration of labelling starts happening and to compare the results of kit labelling studies with those using aqueous DOTA-peptide stock solutions kept frozen at -20 °C for up to 12 months. In addition, some kits were stored at room temperature overnight in order to assess the effect of this possible scenario in the clinical environment on the labelling results.

6.3 Materials and methods

General methods are summarised in chapter 3. This section describes variations and details specific to the work described in this chapter.

6.3.1 Elution of the generator

The ^{68}Ga was eluted from an 1850 MBq (50 mCi) SnO_2-based $^{68}Ge/^{68}Ga$ generator with 0.6 M HCl (see chapter 3). For labelling purposes, fractionated elution was carried out in the following way: 1.5 ml was first eluted to be discarded, followed by a second fraction of 6 ml and finally a third fraction of 2.5 ml which was also discarded. Fractions were collected in sterilized and pre-weighed 10 ml borosilicate glass vials. The middle 6 ml fraction was divided into three 2 ml aliquots, which were each used for labelling of the DOTA-peptide. The

activities in these aliquots ranged from 602 MBq to 111 MBq depending on the age of the generator. ^{68}Ga activities were measured in a dose calibrator.

Elution was also carried out as described in section 5.3.1, elution method 2 in order to assess whether more concentrated, higher activity eluates would yield the same labelling results as the lower activity eluates. The activity contained in the 2 ml fraction ranged from 1006 MBq to 1169 MBq and was used for higher activity labelling of one peptide at a time.

6.3.2 Determination of kit composition parameters, preparation of stock solution and single vial kits

The results obtained following the work described in Chapter 5 were used as a guideline to determine the optimum kit composition parameters required for the preparation of DOTA-peptide kit formulations. These parameters include the optimum quantity of sodium acetate trihydrate required for pH adjustment as well as the required optimum mass of DOTA-peptide to include in the kit. In these labelling experiments DOTA-peptide stock solutions were used, with masses ranging from 15 to 50 µg. It was found that 35 µg of DOTA-peptide was an optimal quantity for radiolabelling DOTA-peptides under the conditions of this study. The pre-determined mass of sodium acetate trihydrate salt required for the DOTA-peptide single vial kits was based on the sodium acetate content of the volume of 2.5 M sodium acetate required to adjust the pH of 2 ml 0.6 M HCl to a pH range of 3.5 to 4 suitable for DOTA-peptide labelling.

DOTATATE, DOTATOC AND DOTANOC stock solutions (1 mg/ml) were prepared as in 3.3.2. Single vial kits were prepared by mixing 35 µl of the thawed DOTA-peptide stock solutions (DOTATATE, DOTATOC and DOTANOC), containing 35 µg of DOTA-peptide, with a pre-calculated and pre-weighed mass of 195.5 mg sodium acetate trihydrate salt in sterile 10 ml borosilicate glass vials. The unsealed single vial kits were individually vacuum-dried under sterile conditions in a desiccator connected with a N 726 FT.18 vacuum pump (KNF Neuberger Inc., Trenton, USA) for 3 hours at room temperature. The vials were then sealed with rubber stoppers and aluminium caps and stored in a freezer compartment at -20 °C for up to 12 months. Frozen DOTA-peptide stock solutions and single vial kits were removed from the freezer and brought to room temperature before being used in each labelling process. Three single vial kits (one of each DOTA-peptide) which had been stored for 12 months at -20 °C, were removed from the freezer and stored at room temperature overnight to assess the effect of this likely scenario in the clinical environment on the labelling results.

6.3.3 *Radiolabelling of the three DOTA-peptide ligands and post-purification process*

When using the DOTA-peptide kit, the 2 ml ^{68}Ga eluate was added to the de-capped 10 ml vial containing the DOTA-peptide kit, which was then sealed again with a sterile rubber stopper and an aluminium cap. When using the DOTA-peptide stock solution, the 2 ml ^{68}Ga eluate was added to a 10 ml vial, followed by 575 µl of 2.5 M sodium acetate solution and then 35 µl of DOTA-peptide stock solution (1 mg/ml). In both cases the mixture was homogenised for 5 seconds by means of gentle swirling in a vortex mixer and the vial containing the labelling mixture was heated for 15 minutes in a controlled heating block at 95°C. The radiolabelled DOTA-peptide reaction mixture was removed from the heating block and homogenised again by means of gentle swirling in a vortex mixer. An aliquot was removed for HPLC testing before the reaction mixture was transferred onto a purification unit consisting of a 500 mg C18, 3 cc, Sep-Pak cartridge. See 3.4.2 for the post-radiolabelling purification process.

6.3.4 *Quality Evaluation of the DOTA-peptide kits and the DOTA-peptide stock solution*

The quality and stability of the DOTA-peptide single vial kits, as well the DOTA-peptide stock solution was evaluated at monthly intervals over a 12-month period as described below.

6.3.4.1 *Radiolabelling efficiency and radiochemical yield*

Radiolabelling efficiency or labelling efficiency (LE) was obtained from the HPLC analysis of an un-purified labelling mixture as described in chapter 3. Radiochemical purities of purified ^{68}Ga-labelled DOTA-peptides were determined by means of HPLC.

For radiochemical yield, refer to 3.4.2. The activities in the collected fractions (**S1** and **S2**), as well as the remaining activities in the reaction vial and on the Sep-Pak C18 cartridge were measured in a dose calibrator.

Radiolabelling efficiency and radiochemical yields of the ^{68}Ga-labelled DOTA-peptides from the single vial kits and stock solutions were monitored over a one-year period.

6.3.4.2 *Microbiological evaluation*

Sterility testing on the DOTA-peptide stock solution and kits was performed before labelling according to the methods prescribed in the European Pharmacopeia (2013). A total of six

samples of the DOTA-peptide single vial kits and six aliquots of 35 µl DOTA-peptide stock solution were tested. These samples (DOTA-peptide kits and 35 µg DOTA-peptide stock solution dissolved in sterile 2 ml 0.6 M HCl) were analysed for bacterial growth. The sterile 2 ml 0.6 M HCl was used instead of 2 ml 0.6 M HCl ^{68}Ga eluate to closely represent the eluate that would be added to the kit but avoid false negative results that may occur due to the presence or the ^{68}Ga. See 3.4.4 for further details of the sterility testing.

Endotoxin testing was performed on the ^{68}Ga eluate, the DOTA-peptide kit and 35 µg DOTA-peptide stock solution dissolved in sterile 0.6 M HCl according to the European Pharmacopoeia (2013) using the chromogenic method (see 3.4.5).

6.3.4.3 pH

The pH value of the DOTA-peptide kits dissolved in 2 ml 0.6 M HCl eluate was determined using pH indicator strips (Merck, Darmstadt, Germany). For the DOTA-peptide stock solutions, the pH value of the 2 ml 0.6 M HCl eluate mixed with 35 µl DOTA-peptide stock solution and 575 µl 2.5 M sodium citrate solution was determined.

6.4 Results

6.4.1 Pre-studies

The activity in the aliquots used in the radiolabelling experiments ranged from 111 MBq to 602 MBq. The radiolabelling results obtained with the different peptide masses showed that the radiolabelling efficiency with 15 µg of DOTA-peptide were less than 85%, the radiolabelling efficiencies with 35 µg were consistently higher than 95 % and the results with 50 µg were not significantly higher than 95 % (see Chapter 5). Based on these results, 35 µg of DOTA-peptide was used in the formulation of the kits and for all the radiolabelling experiments. The inclusion of 195.5 mg sodium acetate trihydrate salt as buffer in the kit formulation ensured a radiolabelling mixture (after the addition of 2 ml ^{68}Ga eluted from the generator with 0.6 M HCl) with a consistent pH of 3.5 – 4.0. The radiolabelling process of heating the reaction mixture in a controlled heating block at 95 °C for 15 minutes resulted in a consistent radiolabelling efficiency above 95 % when the optimum DOTA-peptide mass and sodium acetate buffer was used.

6.4.2 Quality Evaluation of the DOTA-peptide kits and the DOTA-peptide stock solution

6.4.2.1 Radiolabelling efficiency and radiochemical yield

During the 12-month period, if all three peptides are considered together, there was a statistically significant difference in the radiolabelling efficiencies (determined by HPLC) for the DOTA-peptide kits compared to the stock solutions ($p = 0.01$). The difference between the DOTATATE and DOTATOC kits compared to the stock solutions was significant, however the difference between the radiolabelling efficiencies of the DOTANOC kits and stock solutions was not ($p = 0.08$) (see Figure 6.1). At 11 – 12 months, radiolabelling efficiency results on pre-purified ^{68}Ga-labelled DOTA-peptides, obtained from DOTA-peptide single vial kits were consistently above 95 %, with the exception of one result (93 % for DOTANOC) (see Table 6.1). Similar results were obtained when using DOTA-peptide stock solutions. The average radiolabelling efficiency of ^{68}Ga-labelled DOTA-peptides obtained from the DOTA-peptide single vial kits at the end of the 12-month storage period was the same as the average obtained from the DOTA-peptide stock solution after the same period (96 %) (see Figure 6.2).

Table 6.1: Comparison of radiolabelling efficiency of DOTA-peptide kits and stock solutions

HPLC pre-purification radiolabelling efficiency results are shown for ^{68}Ga-DOTA-peptides obtained from DOTA-peptide single vial kits and DOTA-peptide stock solutions, stored for various periods at -20 ˚C

	DOTA-peptide kits			**DOTA-peptide stock solution**		
Peptide Storage Period	**Radiolabelling efficiency (%)**					
	DOTATATE	**DOTATOC**	**DOTANOC**	**DOTATATE**	**DOTATOC**	**DOTANOC**
1-2 months	99.5 (n=4)	99.8 (n=4)	100 (n=4)	95.5 (n=2)	96.3 (n=3)	95.5 (n=2)
3-4 months	98.3 (n=4)	99.5 (n=4)	99.5 (n=4)	97.8 (n=4)	97.7 (n=3)	97.0 (n=2)
5-6 months	100 (n=4)	98.8 (n=4)	95.0 (n=4)	97.0 (n=3)	96.3 (n=4)	98.3 (n=3)
7-8 months	98.7 (n=3)	99.8 (n=4)	98.7 (n=3)	96.5 (n=3)	97.0 (n=3)	98.5 (n=3)
9-10 months	98.5 (n=2)	99.0 (n=2)	96.0 (n=2)	95.0 (n=2)	97.0 (n=2)	96.0 (n=2)
11-12 months	96.5 (n=2)	97.7 (n=3)	93.0 (n=3)	96.0 (n=2)	96.0 (n=2)	97.3 (n=3)

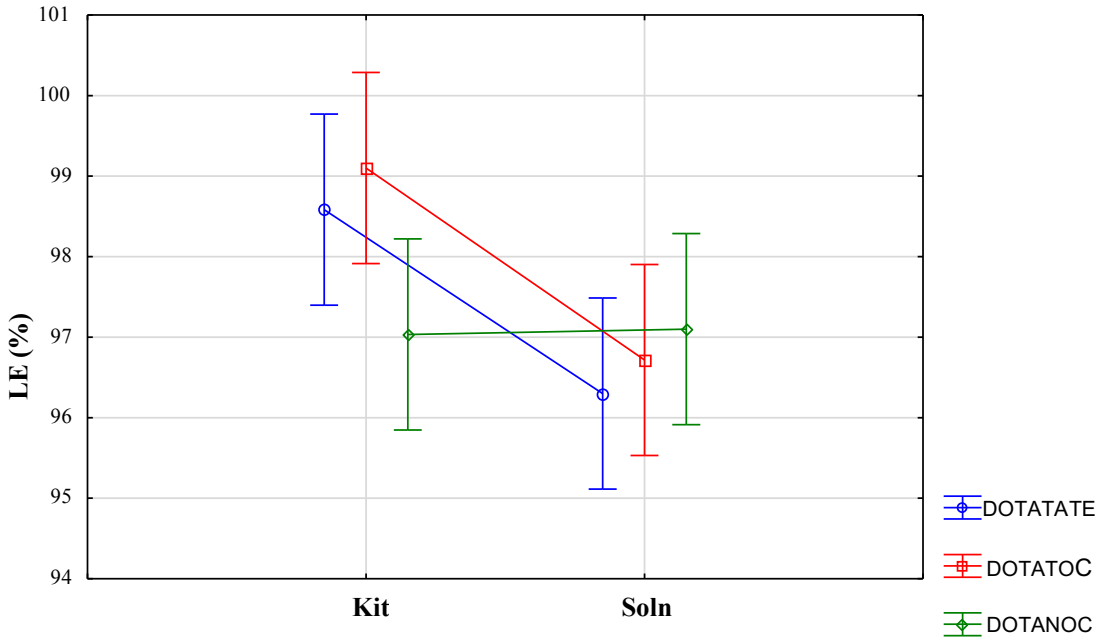


Figure 6.1: Radiolabelling efficiency profile of DOTA-peptide kits and stock solutions

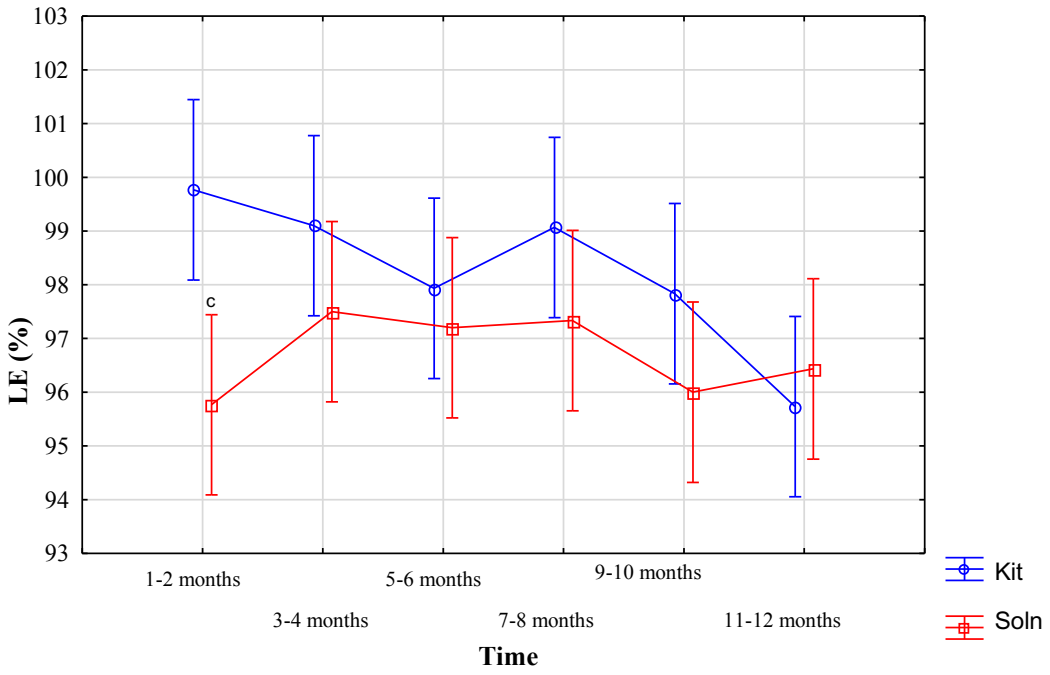


Figure 6.2: Radiolabelling efficiency profile of DOTA-peptide kits and stock solutions during a 12-month period

Statistically there was a significant difference between the radiochemical yields obtained from the DOTA-peptide kits compared to the DOTA-peptide stock solutions over the 12-month evaluation period ($p = 0.02$) (see Figure 6.3). Radiochemical yields with the single vial

DOTA-peptide kits ranged from 73 – 83 % and were similar for all three DOTA-peptides (see Table 6.2). No obvious decline in the radiochemical yields was observed over a 12-month kit storage period. Radiochemical yields obtained using stock solutions of 35 µg DOTA-peptide ranged from 64 – 79 % (see Table 6.3). The average radiochemical yield obtained from the kits after a 12-month storage period was 76 % compared to 67 % for the DOTA-peptide stock solution after the same storage period (see Table 6.4).

The radiochemical purity post C18 purification was always in the order of 100 % (not tabulated).

Table 6.2: Sep-Pak C18 radiochemical yields of ^{68}Ga-DOTA-peptides obtained from single vial kits containing 35 µg DOTA-peptide, stored for various periods at -20 ˚C

Kit Storage Period at -20 ˚C	Radiochemical Yield (%)			Activity lost in reaction vial (%)		
	DOTATATE	DOTATOC	DOTANOC	DOTATATE	DOTATOC	DOTANOC
1-2 months	77 (n=4)	77 (n=4)	73 (n=4)	9 (n=4)	8 (n=4)	9 (n=4)
3-4 months	82 (n=4)	74 (n=4)	79 (n=4)	9 (n=4)	11 (n=4)	8 (n=4)
5-6 months	78 (n=4)	80 (n=4)	73 (n=4)	8 (n=4)	7 (n=4)	9 (n=4)
7-8 months	79 (n=3)	83 (n=4)	77 (n=3)	8 (n=3)	7 (n=3)	9 (n=3)
9-10 months	76 (n=2)	74 (n=2)	80 (n=2)	12 (n=2)	12 (n=2)	9 (n=2)
11-12 months	74 (n=2)	75 (n=3)	78 (n=3)	14 (n=2)	13 (n=3)	8 (n=3)

The average radiolabelling efficiency obtained from three of the kits, which had been stored for 12 months at -20 °C, and then overnight at room temperature, was 98 ± 3 % and the average radiochemical yield was 72 ± 6 % (results not displayed in table form).

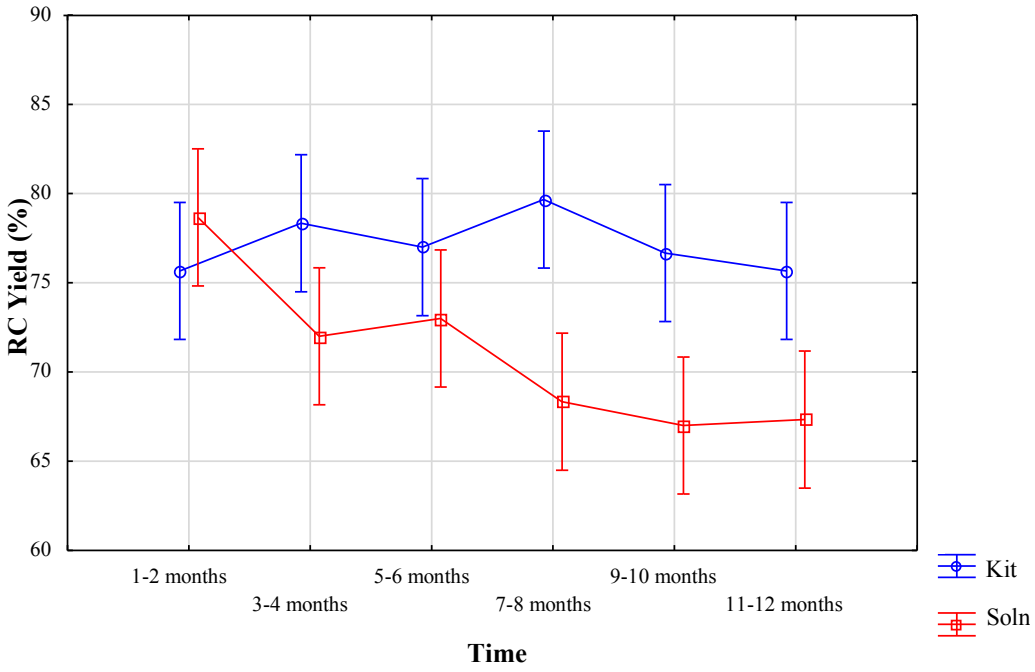


Figure 6.3: Radiochemical yield profile of DOTA-peptide kits and stock solutions

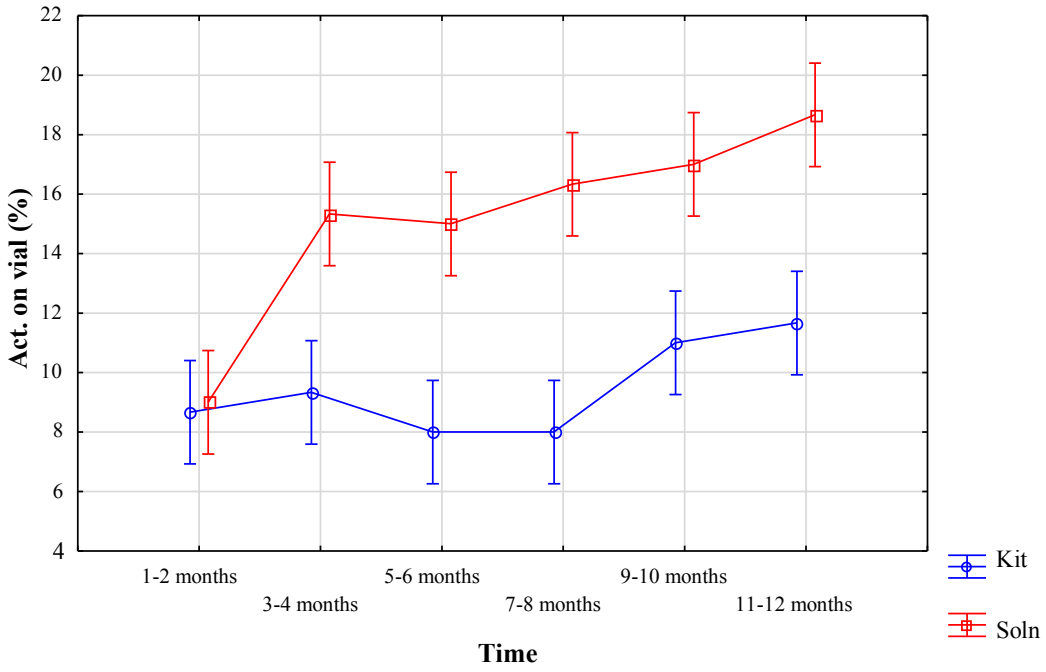


Figure 6.4: Activity remaining on vial after radiolabelling with DOTA-peptide kits and stock solutions

Table 6.3: Sep-Pak C18 radiochemical yields of ^{68}Ga-DOTA-peptides obtained with 35 µg DOTA-peptide stock solution

Age of stock solution at -20 ˚C	Radiochemical Yield (%)			Activity lost in reaction vial (%)		
	DOTATATE	DOTATOC	DOTANOC	DOTATATE	DOTATOC	DOTANOC
1-2 months	79 (n=2)	79 (n=3)	78 (n=2)	11 (n=2)	8 (n=3)	8 (n=2)
3-4 months	69 (n=4)	71 (n=3)	76 (n=2)	18 (n=4)	13 (n=3)	15 (n=2)
5-6 months	67 (n=3)	75 (n=3)	77 (n=3)	16 (n=3)	14 (n=3)	15 (n=3)
7-8 months	66 (n=2)	69 (n=3)	70 (n=2)	16 (n=2)	16 (n=3)	17 (n=2)
9-10 months	64 (n=2)	69 (n=3)	68 (n=2)	20 (n=2)	15 (n=3)	16 (n=2)
11-12 months	67 (n=3)	68 (n=3)	67 (n=3)	20 (n=3)	19 (n=3)	17 (n=3)

6.4.2.2 Microbiological evaluation

All the samples were found to be clear and sterile with no anaerobic bacterial, aerobic bacterial or fungal growth. The endotoxin content of the ^{68}Ga eluates was 1.12 – 1.66 IU/ml, the endotoxin content of the radiolabelled DOTA-peptide kits was 1.02 – 1.09 IU/ml (with an average of 1.05 IU/ml) and that of the radiolabelled DOTA-peptide stock solution was 1.36 – 1.97 IU/ml (with an average of 1.65 IU/ml) (see Table 6.4). There was no inhibition of the reaction because the maximum dilution volume was determined to be 10. The European Pharmacopoeia (2013) specification for bacterial endotoxin is less than 175 IU/V, where V is the maximum volume to be used for the preparation of a single patient dose, if intended for use in the manufacture of parenteral preparations without a further procedure for the removal of bacterial endotoxins. The total volume of the solution (**S2**) post-purification is 3.55 ml.

6.4.2.3 pH

The pH values of the DOTA-peptide kit radiolabelling solution as well as the pH of the DOTA-peptide stock radiolabelling solutions made up with 2 ml ^{68}Ga eluate were all between 3.5 and 4.0.

A summary of the quality control results between DOTA-peptide single vial kits and DOTA-peptide stock solutions after respective storage periods of 12 months at -20 °C is given in Table 6.4.

Table 6.4: Averages of the combined quality control results of three DOTA-peptides, DOTATATE, DOTATOC, DOTANOC, comparing results obtained from single vial kits with those from DOTA-peptide stock solutions after respective storage periods of 12 months at -20 °C

Quality Control Parameters	DOTA-peptide kits	DOTA-peptide stock solution
Radiolabelling Efficiency (%)	96	96
Radiochemical Yield (%)	76	67
Activity lost in reaction vial (%)	12	19
Endotoxin Count (IU/ml)	1.05	1.65
Sterility	Sterile	Sterile
pH	3.5 – 4.0	3.5 – 4.0

6.5 Discussion

In most of the currently available literature studies on DOTA-peptide kit labelling, a titanium dioxide (Eckert & Ziegler) or a nanoceria-PAN based generator was employed as the source of ^{68}Ga, using 0.1 M HCl as the eluent. In some of these studies (Mukherjee et al, 2014b; Das et al., 2014), post-elution processing procedures such as cation exchange purification were also employed. Furthermore, various types of buffer agents such as HEPES, ammonium acetate or sodium acetate were used. Ammonium acetate was found to be unsuitable because the pH changed after lyophilisation of the formulation (Mukherjee et al., 2014a). Kit stability was evaluated over a maximum period of only 6 months (Mukherjee et al., 2014b) and found to be stable with no significant decrease in the radiochemical yields.

This study focused on DOTA-peptide kits, which were formulated specifically for radiolabelling with ^{68}Ga eluted with 0.6 M HCl from a SnO_2-based ^{68}Ge/^{68}Ga generator. Furthermore, this kit-based approach focused more on improving the user-friendliness of the labelling procedure rather than following the approach followed in ^{99m}Tc radiopharmaceutical kit formulations in which heating and post-labelling purification steps are generally not required. A heating step would generally always be required for labelling DOTA-peptides with ^{68}Ga, while the latter would be required to remove ^{68}Ge that might be present in non-purified ^{68}Ga eluates. The DOTA-peptide kits in this study were developed based on results from pre-formulation studies (see Chapter 5) and on radiolabelling processes adapted for the

larger volume and more acidic ^{68}Ga eluates, using fractionated generator eluates. While various buffers such as HEPES have been used in DOTA-peptide radiolabelling studies (Mukherjee et al., 2014b), other investigators (Hoffman et al., 2001; Henze et al., 2001; Breeman et al., 2005; De Blois et al., 2011; Rossouw and Breeman, 2012; Decristoforo et al., 2007; Mukherjee et al., 2014a, 2014c; Das et al., 2014) used acetate buffer in their DOTA-labelling experiments. This buffer type was therefore preferred for this study. The results obtained in Chapter 5 show that 575 µl of a 2.5 M sodium acetate buffer solution was appropriate to adjust the pH of 2 ml 0.6 M HCl eluate to a pH range of 3.5 – 4.0. The stated volume of acetate buffer solution contains 195.5 mg sodium acetate trihydrate salt, which was therefore the quantity used in the kit formulation. The use of eluate volumes in excess of 2 ml was not investigated in this study, as this would result in a lower pH due to the kit design and would therefore most probably result in lower labelling efficiencies. Higher activity can be introduced into labelling mixtures by rather using eluates with higher activity concentration, as shown in Chapter 5. The results described in Chapter 5 also indicated that 35 µg DOTA-peptide resulted in labelling efficiencies consistently above 95 % when used in the volume range of 2.0 – 2.6 ml. This quantity of DOTA-peptide was therefore used in the kit formulation and was added from a pre-prepared 1 mg/ml stock solution to pre-weighed acetate salt. As the kit formulation contained only a minimal volume of water (35 µl from the peptide stock solution), each single vial kit was only vacuum dried for a relatively short period prior to storage at -20 °C compared to the kit studies above (Mukherjee et al., 2014a, 2014b, 2014c) which employed lyophilisation instead. The sodium acetate buffer appeared to remain stable during and after the vacuum drying process (as suggested by the pH results displayed in Table 6.4), unlike in the study above (Mukherjee et al., 2014b) where HEPES was used as a buffer due to the instability of both ammonium acetate (Mukherjee et al., 2014a, 2014b) and sodium acetate (Mukherjee et al., 2014b) buffer both during and after lyophilisation. The kit-based radiolabelling process entailed a post-labelling purification on a Sep-Pak C18 reverse phase cartridge in order to compare radiochemical yields with those obtained using the more conventional stock solution approach. Although the radiochemical purities of labelling mixtures before C18 purification (as reflected by the labelling efficiency results) were generally high, post labelling purification was still required in order to remove traces of ^{68}Ge that are generally still present in fractionated ^{68}Ga eluates. Post labeling purification in kit labelling can only be omitted when using pre-purified eluates. The kits were evaluated over an extended 12-month period, which according to the investigator's knowledge, is the longest evaluation period reported for in-house DOTA-peptide kit preparation.

The radiolabelling and quality control results achieved from the single vial DOTA-peptide kits compared favourably and, in some aspects, were even superior to those achieved from the DOTA-peptide stock solution. The superior average radiochemical yield for the DOTA-peptide kits after a 12-month storage period (76 % compared to 67 % for the DOTA-peptide stock solution) can largely be attributed to the difference in the activity lost in the reaction vial for the DOTA-peptide kits compared to the DOTA-peptide stock solution ($p = 0.006$) (see Table 6.4 and Figure 6.4). The reason for the lower activity loss during kit labelling is not entirely clear, but it might be attributed to a slightly higher stability of the DOTA-peptide in the kit formulation than in the frozen stock solution. These results demonstrate that the single vial DOTA-peptide kits are stable for at least up to 12 months. In the likely clinical scenario where the single vial kits may be un-intentionally left outside the freezer overnight (at room temperature), the average radiolabelling efficiency of 98% and the average radiochemical yield of 72 % demonstrated that the radiolabelling results should not be adversely affected by such an action. The average endotoxin count for the DOTA-peptide kits was also lower (1.05 IU/ml) compared to the DOTA-peptide stock solution (1.65 IU/ml). Based on the microbiological evaluation results, the radiolabelled DOTA-peptide kits were found to be sterile, pyrogen-free and microbiologically suitable for clinical intravenous application according to the European Pharmacopoeia (2013) specifications.

The pH of the DOTA-peptide kits and stock solutions were both between 3.5 and 4.0, which fall within the acceptable pH range required for the DOTA-peptide labelling process.

Further improvements to the radiolabelling procedure can be made for quality assurance in a clinical environment. These improvements may include adding the ^{68}Ga eluate to the peptide by piercing the stopper (instead of de-capping the vial) as well as replace the mixing by vortex with a gentle swirling action.

6.6 Conclusion

After fractional elution, the use of DOTA-peptide single vial kits offers a significant advantage in terms of convenience, stability and user-friendliness of the radiolabelling procedure. The kit manufacturing procedure described in this study is relatively simple and does not require a lyophilisation step. An added advantage is the reduced activity lost during C18 purification of the [^{68}Ga]-DOTA-peptide, resulting in slightly higher radiochemical yields. The use of the labelling kit formulations (using the fractionated elution method) appears to be highly feasible and the quality of the DOTA-peptide kits was found to be

superior over DOTA-peptide stock solutions, as reflected by the radiochemical yield results obtained after an extended kit storage period.

Chapter 7: Concentration and purification of ^{68}Ga eluates on cation exchange resins

7.1 Introduction

Despite the favourable radiolabelling results obtained by using fractionated ^{68}Ga eluates (see Chapter 5), the relatively large volumes and high concentration of HCl in the fractionated eluates (especially when using a SnO_2-based generator as source of ^{68}Ga) still complicate the direct use thereof in the labelling reactions. In some instances, the use of pre-labelling purification of eluates might also be preferred over the post-labelling purification techniques to remove traces of ^{68}Ge contaminants. Different alternative pre-labelling processing techniques of ^{68}Ga eluates have been developed for radiolabelling DOTA-conjugated peptides. They have been described for either semi- or fully automated systems. These methods are based on pre-purification and concentration of the ^{68}Ga eluate using either anion exchange resins, cation exchange resins or combined cationic/anionic resin purification. Anion exchange purification can be somewhat cumbersome as explained in Section 1.7.1, while the same could apply to a combined anionic/cationic technique. According to Mueller et al. (2012), the cation exchange technique appears to be more favourable. This chapter therefore deals exclusively with the use of cation exchange resins.

In the cation exchange concentration and purification method described by Zhernosekov et al. (2007), the 0.1 M HCl ^{68}Ga eluate (from a TiO_2-based generator) is loaded onto a cation exchange resin. Due to the high distribution coefficient, the ^{68}Ga is adsorbed on the resin. The column is then eluted with HCl/acetone mixtures (80 % acetone and 0.1 – 0.2 M HCl) to remove the Ge^{4+}, Ti^{4+}, Zn^{2+} and Fe^{3+} while the ^{68}Ga remains on the resin and is subsequently eluted with 400 µl of a 97.6 % acetone/0.05 M HCl solution. This cation-exchange purification method results in smaller ^{68}Ga eluate volumes and removes almost all chemical and radiochemical impurities including ^{68}Ge. The purified ^{68}Ga eluate can be used for direct labelling. A drawback of this method is that it requires the use of acetone, which has to be removed from the final formulation because acetone is not approved for intravenous use.

Mueller et al. (2012) described an alternative, user-friendlier cation exchange concentration/purification method for ^{68}Ga eluates obtained from a TiO_2 generator. This method was based on the use of an acidified aqueous sodium chloride (NaCl) solution instead of acetone as the desorption agent, using a silica-based strong cation exchange (SCX) cartridge. A subsequent labelling method was also described that enables high-efficiency labelling of DOTA conjugated peptides in high radiochemical purity (Mueller et al., 2012).

The Mueller method used eluates containing 0.1 M HCl, which were directly loaded on the SCX resin.

Martin et al. (2014) tested and compared various commercial cation exchange cartridges for 0.6 M HCl ^{68}Ga eluates from a SnO_2 generator adapting the Mueller et al. (2012) method. They found that the Chromafix PS-H^+ (size M) gave the best overall results for the ^{68}Ga-trapping efficiency and the efficiency of subsequent ^{68}Ga elution from these cartridges.

This chapter describes investigation into various aspects of the work of Mueller et al. (2012) and Martin et al. (2014) in order to identify the best cationic resin and conditions for the cationic resin purification of ^{68}Ga eluates obtained from a SnO_2-based generator containing 0.6 M HCl.

7.2 Aim

The aim of this study was to investigate the concentration and purification of ^{68}Ga eluates from a SnO_2-based generator on different cation exchange resins, including a silica-based Bond Elut SCX 100 mg resin. The ultimate aim was to identify the resin that can optimally retain ^{68}Ga in 0.6 M HCl as well as to be able to optimally release the ^{68}Ga with the desorption mixture that was described in the Mueller et al. (2012) method. Alternatively, if no resin is found to be suitable to fulfil both these criteria, methods would be investigated on how to adapt the Mueller method, using eluates in 0.6 M HCl.

7.3 Materials and methods

General methods are summarised in chapter 3. This section describes variations and details specific to the work described in this chapter.

7.3.1 Elution of the generator

The ^{68}Ga was eluted from an 1110 MBq (30 mCi) SnO_2-based ^{68}Ge/^{68}Ga generator with 0.6 M HCl (see chapter 3). The elution was carried out with 12 ml 0.6 M HCl for the comparative resin experiments (see Table 7.1) and 10 ml 0.6 M HCl for the specific SCX resin experiments (see Table 7.2 and Figure 7.1). Eluates were collected in borosilicate glass vials.

7.3.2 *Types of cation exchange resins used*

Comparative experiments were performed using three different commercially available cationic resin cartridges (Bond Elut SCX from Agilent Technologies, 100 mg sorbent, Chromafix PS-H^+ (S) from Machery-Nagel, average 230 mg sorbent, and the Phenomenex Strata-X-C Polymeric Strong Cation 100 mg/3 ml) as well as four alternative cationic resin cartridges prepared in-house (AG 50W-X2, AG 50W-X4, AG 50W-X8 and AG MP-50) (see also 7.3.3).

7.3.3 *Preparation of alternative cation exchange resin cartridges*

Cartridges of the cationic exchange resins, AG 50W-X2 (catalogue number 1421241), AG 50W-X4 (catalogue number 1421341), AG 50W-X8 (catalogue number 1421451) and AG MP-50 (catalogue number 1430841) (all from Bio-Rad, California, United States), were in-house prepared as follows:

Four used Bond Elut SCX 100 mg (1 ml) cartridges, catalogue number 12102013, (Agilent Technologies, Santa Clara, United States) were emptied of the resin, rinsed, dried and individually labelled with the different cation exchange resin names. 100 mg of each resin was weighed into the corresponding labelled cartridges.

7.3.4 *Concentration of ^{68}Ga eluates on the cation exchange resins (see also 3.4.10)*

The cation exchange resin cartridges were pre-conditioned as described in chapter 3. For the specific Bond Elut SCX resin experiments (see Table 7.2 and Figure 7.1), the 10 ml 0.6 M HCl ^{68}Ga eluates were either divided into smaller aliquots, which were loaded directly on the resin cartridge in separate experiments, or diluted with ultrapure pharmaceutical grade water to pre-determined HCl concentrations, then divided into suitable smaller aliquots which were subsequently loaded on the resin in separate experiments. For the comparative resin experiments (see Table 7.1), the 12 ml 0.6 M HCl ^{68}Ga eluates were not diluted, but divided into three 4 ml aliquots for separate experiments. The 4 ml ^{68}Ga eluate was loaded onto the pre-conditioned resin cartridge and eluted into a waste vial (**Fr1).** The resin was run dry and eluted with 1 ml water into the same waste vial. This vial was replaced with another vial and the ^{68}Ga was eluted from the resin with 0.5 ml of a mixture of 5 M NaCl and 5.5 M HCl 40:1 (v/v). The resin was run dry into the same vial (**Fr2**) and the activities of **Fr1** and **Fr2** as well as the residual activity on the resin cartridge were determined**.** Refer to 3.4.10 for additional details.

7.4 Results

The results in Table 7.1 present data on the retention of ^{68}Ga activity in 0.6 M HCl medium on different cation exchange resins, as reflected by the eluted activity in the waste vial (**Fr1**). Low activity in the waste implies greater retention of ^{68}Ga on the resin, while higher activity implies poorer retention. Data on the subsequent recovery of activity in **Fr2**, using 0.5 ml of a mixture of 5 M NaCl and 5.5 M HCl 40:1 (v/v), is also given. The activity in the waste vial **Fr1** (average values) was the lowest (2 %) for AG 50W-X8 and Chromafix PS-H$^+$ (S), and highest (88 %) for Strata-X-C.

The highest ^{68}Ga activity recovered from the cation exchange resin cartridge (in the second eluate fraction **Fr2**) (average values) was for the Bond Elut SCX (80 ± 1 %). The lowest ^{68}Ga activity (4%) in **Fr2** was for Strata-X-C due to its low capacity to trap Ga^{3+} ions.

Table 7.1: Comparison of eluate purification performance of different cation exchange resins

Data on the retention of ^{68}Ga activity in 0.6 M HCl on different cation exchange resins as well as the subsequent recovery of activity using 0.5 ml of a mixture of 5 M NaCl and 5.5 M HCl 40:1 (v/v)

Type of cation exchange resin	Activity in waste Fr1 (%)	Recovered ^{68}Ga in Fr2 (%)	Residual Act. in cation exchange resin (%)
AG 50W-X2 100 mg	56	15	29
	56	15	28
	64	10	26
AG 50W-X4 100 mg	26	35	44
	25	34	41
	30	30	42
AG 50W-X8 100 mg	2	33	67
	2	37	63
	2	34	68
AG MP-50 100 mg	3	60	36
	6	45	50
	9	45	47
Bond Elut SCX 100 mg	19	80	1
	19	79	2
	18	80	2
Chromafix PS-H$^+$ (S) 230 mg	1	54	45
	2	58	40
	3	51	46
Strata-X-C 100 mg	88	4	8
	88	4	8
	88	4	8

The activity remaining on the cation resin (average values) was the lowest for the Bond Elut SCX cartridge (2 ± 0.6 %), followed by Strata-X-C (8 %), then AG 50W-X2 (28 ± 1.5 %), followed by the AG 50W-X4 (42 ± 1.5 %), slightly higher for both the AG MP-50 and Chromafix PS-H^+ (S), (44 ± 7 % and 44 ± 3 % respectively) and highest for the AG 50W-X8 (66 ± 3 %).

The results in Table 7.2 and Figure 7.1 present data on the influence of HCl molar concentration and volume of ^{68}Ga-containing load solutions in HCl on the retention of ^{68}Ga activity on the Bond Elut SCX resin. Table 7.2 also presents data on the subsequent recovery of activity using 0.5 ml of a mixture of 5 M NaCl and 5.5 M HCl = 40:1 (v/v), as illustrated by the activity in **Fr2**.

In 0.6 M HCl, the eluted activity in the waste vial (**Fr1)** increased from 2.5 % to 18.4 % as the volume of diluted ^{68}Ga aliquot increased from 2 to 4 ml and the activity in **Fr2** decreased from 95.8 % to 79.9 % accordingly. The activity retained on the Bond Elut SCX cartridge did not exceed 1.5 %.

In 0.5 M HCl, the eluted activity in the waste vial (**Fr1**) increased from 1.5 % to 6.7 % as the volume of diluted ^{68}Ga aliquot increased from 3 to 5 ml and the activity in **Fr2** decreased only slightly from 95.8 % to 91.3 %. The activity retained on the Bond Elut SCX cartridge decreased from 2.4 % to 1.8 %

In 0.4 M HCl, the eluted activity in the waste vial (**Fr1)** remained fairly constant (from 0.2 to 0.3 %) as the volume of diluted ^{68}Ga aliquot increased from 4 to 6 ml and the activity in **Fr2** remained fairly constant and decreased only slightly from 97.5 % to 96.2 %. The activity retained on the Bond Elut SCX cartridge also remained fairly constant and only decreased slightly from 2.1 % to 2 %.

In 0.3 M HCl, the eluted activity in the waste vial (**Fr1**) remained constant (0.4 %) as the volume of diluted ^{68}Ga aliquot increased from 2 to 6 ml and decreased to 0.2 % in a volume of 8 ml. The activity in **Fr2** consistently increased slightly from 96.5 % to 97.8 % as the volume of diluted ^{68}Ga aliquot increased from 2 to 8 ml. The activity retained on the Bond Elut SCX cartridge decreased consistently from 2.4 % to 1.6 % as the volume of diluted ^{68}Ga aliquot increased.

Table 7.2: Effect of HCl concentration and volume on eluate purification

Data on the retention of ^{68}Ga activity in HCl on a Bond Elut SCX cartridge as a function of HCl concentration and volume of ^{68}Ga/HCl load solution as well as the subsequent recovery of activity using 0.5 ml of a mixture of 5 M NaCl and 5.5 M HCl 40:1 (v/v)

Vol. of diluted ^{68}Ga aliquot (ml)	HCl conc. after dilution (M)	Activity in waste vial Fr1 (%)	Recovered ^{68}Ga in Fr2 (%)	Residual Activity in SCX (%)
2	0.6	2.5	95.8	1.1
3	0.6	7.5	90.1	1.5
4	0.6	18.4	79.9	1.0
3	0.5	1.5	95.8	2.4
4	0.5	4.1	93.9	1.8
5	0.5	6.7	91.3	1.8
4	0.4	0.2	97.5	2.1
5	0.4	0.2	97.6	2.0
6	0.4	0.3	96.2	2.0
2	0.3	0.4	96.5	2.4
4	0.3	0.4	97.2	2.0
6	0.3	0.4	97.5	1.8
8	0.3	0.2	97.8	1.6

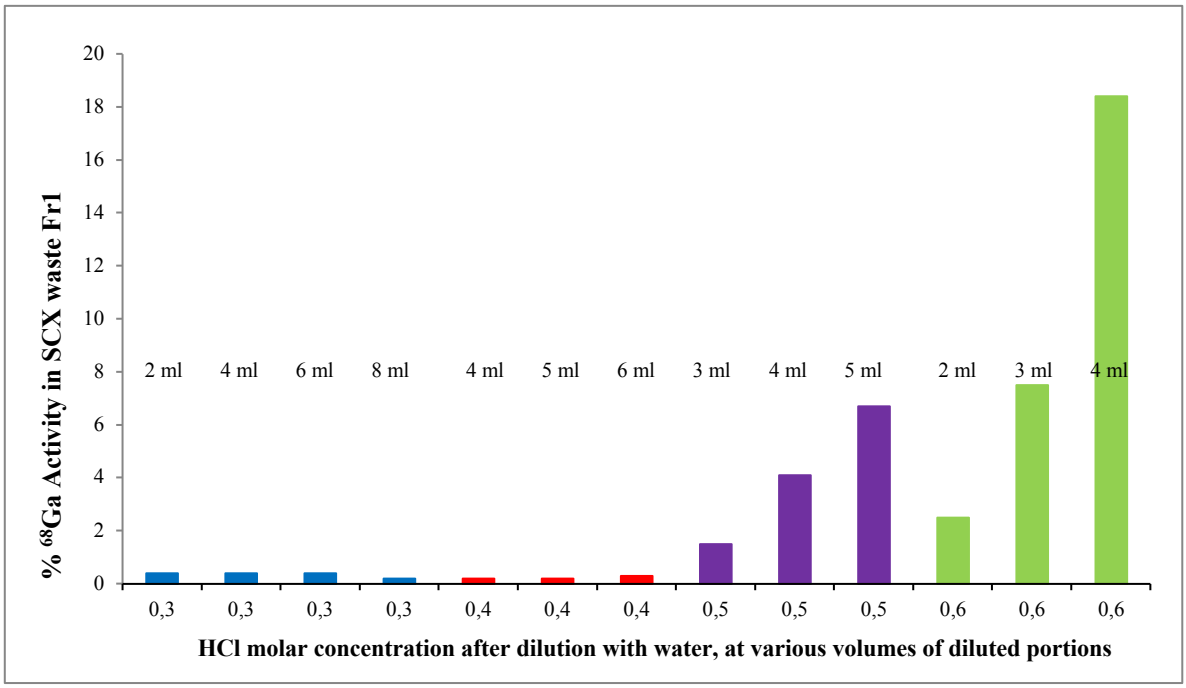


Figure 7.1: Influence of HCl molar concentration and volume of ^{68}Ga-containing load solutions in HCl on the retention of ^{68}Ga activity on a SCX cartridge, as illustrated by the eluted activity in the waste vial **Fr1**. The following load solution volumes (in brackets) were associated with the various HCl concentrations: 0.3 M ■ (2 ml, 4 ml, 6 ml, 8 ml); 0.4 M ■ (4 ml, 5 ml, 6 ml); 0.5 M ■ (3 ml, 4 ml, 5 ml); 0.6 M ■ (2 ml; 3 ml, 4 ml)

7.5 Discussion

Various cation exchange resins (Bond Elut SCX 100mg, Chromafix PS-H^+ (S), Strata-X-C, AG 50W-X2, AG 50W-X4, AG 50W-X8 and AG MP-50) were investigated to assess their suitability for adsorption of ^{68}Ga in 0.6 M HCl, which is the eluent of choice for the elution of ^{68}Ga from a SnO_2-based generator. The adsorption abilities of the resins were assessed by means of the un-retained activity in the waste vial (**Fr1**). Based on the ^{68}Ga activities in the waste vial, the most suitable resins for the adsorption of ^{68}Ga in 0.6 M HCl appear to be AG 50W-X8 resin and Chromafix PS-H^+ (S) cartridges (2 ± 0 % and 2 ± 1 % respectively), while the AG MP-50 resin (6 ± 3 %), the Bond Elut SCX cartridge (19 ± 1 %) and the AG 50W-X4 resin (27 ± 3 %) show less efficient adsorption in this medium. Strata-X-C resin proved to be the least suitable, displaying the most activity in the waste vial (88 %). However, the relatively large residual activity in AG 50W-X8 (66 ± 3 %), AG MP-50 (44 ± 7 %), Chromafix PS-H^+ (S) (44 ± 3 %) and AG 50W-X4 (42 ± 1.5 %) after elution result in lower activities in **Fr2** for these resins (35 ± 2 %, 50 ± 9 %, 54 ± 4 % and 33 ± 2 % respectively), in other words, less ^{68}Ga could be recovered from these columns. Martin et al. (2014) found that the Bond Elut SCX cartridge (100 mg) displayed a low adsorption efficiency of 7 % compared to this study (almost 80 %). They however, used an HCl concentration of 1.0 M as well as bigger volumes (10 ml). They did find that the adsorption of $^{68}Ga^{3+}$ on this cartridge was influenced by different concentrations of HCl, which concurred with this study. They also found that the adsorption yield increased from 7 % to 42 % with a larger quantity of resin (Bond Elut SCX cartridge 500 mg instead of 100 mg). Martin et al. (2014) also used larger Chromafix PS-H^+ cartridges (size M with 430 mg resin) with 1 M HCl (10 ml), which resulted in 69 % adsorption with a single resin cartridge. The Chromafix PS-H^+ (size M) cartridges gave them the best desorption results from seven cation exchange cartridges that they tested. They also found that the adsorption of the $^{68}Ga^{3+}$ on the Chromafix PS-H^+ cartridge increased when the eluate was diluted with water prior to loading the solution onto the cartridge. In contrast, in this investigation 4 ml of 0.6 M HCl was used with Chromafix PS-H^+ (S) cartridges containing 230 mg resin, which resulted in 98 % adsorption. However, in this study the desorption from this resin was found to be only about 54 %, compared to the 94 % reported by Martin (2014), using HCl/NaCl solution. These results demonstrate how the mass of the cationic resin may influence its efficiency at adsorbing and desorbing the ^{68}Ga. Due to the unfavourable results in this investigation, Chromafix PS-H^+ (S) resin was not selected for further investigation in this study.

Based on displaying the highest ^{68}Ga activity recovered from the cation exchange column (in **Fr2)** (accompanied with the lowest residual activity on the resin after collection of **Fr2,** as shown in Table 7.1)**,** the Bond Elut SCX cartridge showed the most promising overall properties for further investigation. This involved finding a means to improve the adsorption ability of the ^{68}Ga activity on the resin (reduction of the amount of activity in waste vial **Fr1**) by lowering the HCl concentration of the eluate.

Considering the combined results in Table 7.1, further investigation of the Bond Elut SCX (100 mg) cartridge was carried out to determine the optimal HCl concentration of the eluate and volume of the diluted ^{68}Ga aliquot to improve the adsorption and subsequent recovery of ^{68}Ga.

In Table 7.2 and Figure 7.1 data are presented of various concentrations of HCl (0.3 – 0.6 M HCl) and volume of ^{68}Ga-containing load solutions in HCl on the retention of ^{68}Ga activity on the Bond Elut SCX cartridge.

From Table 7.2, the best overall results (based on the activity in the waste vial (**Fr1**), **Fr2** and residual activity in the Bond Elut SCX cartridge) were obtained with 0.3M HCl (4 ml of 0.6 M HCl ^{68}Ga aliquot + 4 ml water). This implies that if a SnO_2-based generator is eluted with 4 ml of 0.6 M HCl and the eluate is diluted to 8 ml with water, the eluate can be passed through the SCX resin cartridge with minimal activity loss. From the results obtained, 4 ml of 0.6 M HCl diluted to 6 ml (to render 0.4 M) also resulted in a small loss of activity, which may indicate that either of these dilutions can be used. The 0.3 M HCl and 8 ml of diluted ^{68}Ga aliquot is however preferred. The SCX purification/concentration method under these conditions resulted in the availability of almost 98 % of the ^{68}Ga eluate from the generator for radiolabelling, which concurs with the result (> 98 %) obtained by Mueller et al. (2012).

7.6 Conclusion

A cationic resin-based eluate purification method based on that developed by Mueller et al. (2012) for eluates from a TiO_2 generator, was successfully adapted for the SnO_2-based generator, which is eluted with higher concentrations of HCl, by diluting the eluate. The ^{68}Ga was efficiently adsorbed on a Bond Elut SCX (100 mg) cartridge and then desorbed by acidified solutions of NaCl. It offers the advantage over the acetone-based cationic processes of avoiding the organic solvent during the labelling process.

Chapter 8: Purification of metal-spiked ^{68}Ga eluates on a strong cation exchange (SCX) resin

8.1 Introduction

Radiolabelling procedures with ^{68}Ga are influenced by the presence of certain metals (Zhernosekov et al. 2007). Metal contamination may affect radiolabelling processes that require high specific activities. Velikyan et al. (2008) report the imaging of low-density cell receptor sites requires radiopharmaceuticals with high specific activity. Metals present during the radiolabelling reaction may bind to the peptide ligands, resulting in non-radioactive peptides, which would compete with the ^{68}Ga-labelled peptides for receptor occupancy. Metals may be introduced in the radiolabelling process and their presence may largely result from metal contamination of the HCl eluent or reagents, or ions that may leak from the generator column. Metal contaminants include Zn^{2+}, which is the non-radioactive decay product of ^{68}Ga. Oehlke et al. (2013) investigated the influence of various metal cations on the radiolabelling yield of [^{68}Ga]DOTATATE and found that the metal ion/ligand ratio (and not the metal concentration) plays an important role in labelling yields. They found that the metal ions that have the largest effect were Pb^{2+}, Cu^{2+}, Fe^{3+} and Zn^{2+}. They also found that [^{68}Ga]DOTATATE was stable in the presence of high concentrations of Fe^{3+} and Zn^{2+}, but transmetalates with Cu^{2+} at 95 °C, which exerts the greatest influence on the [^{68}Ga]DOTATATE complexation. They found that the effect of Cu^{2+} showed a pH dependency with a decrease in labelling yield with increasing pH. They showed that complete labelling (100 %) was observed when the metal/ligand ratio was below 1:1. Their results were consistent with previous studies (Šimeček et al., 2013; Velikyan et al., 2008) that Al^{3+} had a very low influence of the $^{68}Ga^{3+}$ complexation with DOTA ligands. They also corroborate the findings of Velikyan et al. (2008) that Fe^{3+} competes strongly with Ga^{3+} in the labelling reaction.

Eluate fractionation or eluate pre-concentration and pre-purification can be used to partially remove unwanted metals. Various ion exchange resins have been used in studies on the purification and concentration of ^{68}Ga eluates. One of the most comprehensive studies on this topic has been described by Zhernosekov et al (2007). They investigated the pre-concentration and purification of the ^{68}Ga eluates from a TiO_2 generator on a Bio-Rad AG 50W-X8 cation exchange resin using HCl/acetone as an eluent. The relative distribution of various metals [Ga^{3+}, Ge^{4+}, Zn^{2+}, Ti^{4+} and Fe^{3+}] in various eluate fractions was determined. They found that the initial quantities of Zn^{2+}, Ti^{4+} and Fe^{3+} were reduced by factors of 10^5, 10^2, and 10, respectively. The ^{68}Ga desorption agent used in this cationic method contains the organic

solvent acetone, which requires additional quality control of the labelled products and could also lead to breakdown impurities in combination with HCl (Mueller et al 2012).

Ocak et al. (2010) investigated the purification of the ^{68}Ga eluate using a Bio-Rad AG 50W-X4 cation exchange resin as well as a Phenomonex Strata-X-C cation exchange cartridge using a TiO_2 generator. They also employed the HCl/acetone method used by Zhernosekov et al. (2007). The Fe, Al and Ti quantities in the eluate were significantly lower for the AG 50W-X4 cation exchange resin compared to the Strata-X-C resin. The Zn and Cu concentrations were however found to be higher.

In order to eliminate the use of acetone, a user-friendly cationic method was developed and described in a study by Mueller et al (2012), using NaCl instead of acetone. The latter study, however, does not include any data on the removal of various metals from eluates, using this system. A slightly modified version of the Mueller method was described in Chapter 7, specifically addressing the concentration/purification of eluates from a SnO_2-based generator. The intent of the investigation in this chapter was therefore to determine the ability of the modified SCX/NaCl/HCl purification technology to remove the most likely metal contaminants from SnO_2-based generator eluates.

8.2 Aim

The aim of this investigation was to establish the ability of the cationic Bond Elut SCX (100 mg) cartridge to remove known quantities of Zn^{2+}, Cu^{2+}, Ga^{3+} and Fe^{3+} which were deliberately introduced into the ^{68}Ga eluate obtained from a SnO_2-based generator. These metal ions were selected based on their most likely presence in the SnO_2 generator. Al^{3+} was not included due to its low influence on the radiolabelling process.

8.3 Materials and Methods

General methods are summarised in chapter 3. This section describes variations and details specific to the work described in this chapter.

The ^{68}Ga was eluted from an 1110 MBq (30 mCi) SnO_2-based ^{68}Ge/^{68}Ga generator with 0.6 M HCl (see 3.4.1).

8.3.1 *Spiking of eluate with metal mixtures containing zinc (Zn), iron (Fe), copper (Cu) and gallium (Ga)*

1000 ppm standards of each metal in 5 % (0.8 M) nitric acid (HNO_3) (catalogue numbers: Zn – 88118, Fe – 88073, Cu – 88061 and Ga – 88066) from Alfa Aesar (Haverhill, Massachusetts, United States) were used. The following mixtures of Cu, Zn, Fe and Ga were prepared:
250 ppm of each metal in 1 ml, 0.08 ml and 0.04 ml mixture of the 4 metals (Zn, Cu, Ga and Fe):
A 250 µg metal mixture was prepared in 1 ml, by combining 0.25 ml of each metal stock solution together in a vial. Addition of 4 ml eluate to this vial, rendered a metal content of 250 µg metal of each metal in 5 ml, i.e. 50 ppm of each metal.
A 20 µg metal mixture was prepared in 0.08 ml, by combining 0.02 ml of each metal stock solution together in a vial. Addition of 4 ml eluate to this vial, rendered a metal content of 20 µg metal of each metal in 4.08 ml, i.e. 4.9 ppm of each metal.
A 10 µg metal mixture was prepared in 0.04 ml, by combining 0.01 ml of each metal stock solution together in a vial. Addition of 4 ml eluate to this vial, rendered a metal content of 10 µg metal of each metal in 4.04 ml, i.e. 2.48 ppm of each metal.

These solutions were further diluted with 5 ml ultrapure pharmaceutical grade water, which resulted in solutions with 25 ppm, 2.2 ppm and 1.11 ppm of each metal in the respective solutions.

8.3.2 *Purification of metal-spiked eluates on Bond Elut SCX cartridge*

A Bond Elut SCX 100 mg (1 ml) cartridge was pre-conditioned as described in chapter 3. The metal-spiked eluate above was mixed by a vortex action to ensure homogeneity and the activity was loaded onto the Bond Elut SCX cartridge and eluted into a borosilicate vial. The resin was run dry and rinsed with 1 ml water into the same waste vial (**Fr1**). This vial was sealed and replaced with another vial. The resin cartridge was loaded with 0.5 ml of a mixture of 5 M NaCl and 5.5 M HCl 40:1 (v/v) and the ^{68}Ga was eluted from the resin. The resin was run dry, the vial (**Fr2**) sealed and the activities in both vials (**Fr1** and **Fr2**), as well as the residual activity in the cartridge, were determined.

8.3.3 Metal analysis of the eluates

The metal contaminants (Zn, Fe, Cu and Ga) in the eluates (**Fr1** and **Fr2**) were determined using a Horiba Jobin Yvon Ultima inductively coupled plasma optical emission spectrometer (ICP-OES). The concentration of metals in the samples was determined from calibration curves ranging from 1 ppm to 25 ppm.

8.4 Results

8.4.1 Metal analysis of the eluates

The results in Table 8.1 and Table 8.2 present data on the metal content in parts per million (ppm) and microgram (µg) respectively, of various SCX fractions after passing diluted metal-spiked ^{68}Ga eluates through a Bond Elut SCX (100 mg) cation resin cartridge and recovery of ^{68}Ga activity with 5 M NaCl/5.5 M HCl 40:1 (v/v) solution. Based on these results, the information below summarises the percentage (%) metals in each of these SCX fractions.

The addition of metal spiked ^{68}Ga eluates, containing 250 µg (25 ppm) of each metal, to the SCX cartridge resulted in the following metal content (expressed as percentage of spiked metal) in the waste vial (**Fr1**) after passing the eluates through the SCX resin: 59 % Fe; 89 % Zn; 76 % Cu and 92 % Ga. The corresponding metal content in **Fr2** was as follows: 0.9 % Fe; 0.6 % Zn; 0.5 % Cu and 0.2 % Ga.

The addition of metal spiked ^{68}Ga eluates, containing 20 µg (2.2 ppm) of each metal, to the SCX cartridge resulted in the following metal content in the waste vial (**Fr1**) after passing the eluates through the SCX resin: 83 % Fe; 90 % Zn; 58 % Cu and 85 % Ga. The corresponding metal content in **Fr2** was as follows: 3.5 % Fe; 2 % Zn; 1.5 % Cu and 0.5 % Ga.

The addition of metal spiked ^{68}Ga eluates, containing 10 µg (1.11 ppm) of each metal, to the SCX cartridge resulted in the following metal content in the waste vial (**Fr1**) after passing the eluates through the SCX resin: 78 % Fe; 100 % Zn; 48 % Cu and 88 % Ga. The corresponding metal content in **Fr2** was as follows: 2 % Fe; 4 % Zn; 1 % Cu and 1 % Ga. The combined metal content in **Fr1** and **Fr2** sometimes exceeded 100 % due to the percentage error in measurement of small quantities of metal.

From the tables, it can be observed that for all three concentrations of metals (10, 20 and 250 µg) that were introduced, consistently an average of 79 % of the total “spiked” metals (Fe,

Zn, Cu and Ga) were eluted in the waste vial (**Fr1**) and only 0.6 – 2 % of the total introduced metals (for all three concentrations of "spiked" metal) were desorbed into **Fr2.** Based on the total of the percentages of the metals in the waste vial (**Fr1**) and **Fr2,** there appeared to be residual quantities of metal retained on the SCX cartridge. The metal content in **Fr2** was therefore used as the indicator for metal removal efficiency and for all three concentrations of "spiked" metal, the SCX resin most effectively removed the Ga, then the Cu and finally the Fe and Zn.

Table 8.1: Removal of metals by SCX cartridge (ppm)

Metal content in parts per million of various SCX fractions measured after passing diluted metal-spiked ^{68}Ga eluates through a Bond Elut SCX 100 mg cartridge and recovery of activity with 5 M NaCl/5.5 M HCl 40:1 (v/v) solution

Metal content of diluted metal-spiked ^{68}Ga eluates before SCX		**Metal content after SCX (ppm)**							
		SCX Fr1 (10 – 11 ml waste)				**SCX Fr2 (0.5 ml 5 M NaCl/5.5 M HCl 40:1)**			
(µg)	**(ppm)**	**Fe**	**Zn**	**Cu**	**Ga**	**Fe**	**Zn**	**Cu**	**Ga**
250	25	13.4 ± 1.4	20.1 ± 0.8	17.1 ± 0.8	21.6 ± 0.6	4.7 ± 0.5	2.8 ± 0.4	2.5 ± 0.1	1.2 ± 0.1
20	2.2	1.7 ± 0	1.8 ± 0.2	1.1 ± 0.1	1.7 ± 0.1	1.3 ± 0.2	0.9 ± 0.1	0.5 ± 0	0.2 ± 0
10	1.11	0.8 ± 0.3	1.0 ± 0.2	0.5 ± 0.1	0.9 ± 0	0.4 ± 0	0.7 ± 0.1	0.2 ± 0	0.2 ± 0

*n = 3

Table 8.2: Removal of metals by SCX cartridge (µg)

Metal content in microgram of various SCX fractions measured after passing diluted metal-spiked ^{68}Ga eluates through a Bond Elut SCX 100 mg cartridge and recovery of activity with 5 M NaCl/5.5 M HCl 40:1 (v/v) solution

Metal content of diluted metal-spiked ^{68}Ga eluates before SCX		**Metal content after SCX (µg)**							
		SCX Fr1 (10 – 11 ml waste)				**SCX Fr2 (0.5 ml 5 M NaCl/5.5 M HCl 40:1)**			
(µg)	**(ppm)**	**Fe**	**Zn**	**Cu**	**Ga**	**Fe**	**Zn**	**Cu**	**Ga**
250	25	147 ± 15	222 ± 8	189 ± 9	230 ± 9	2.3 ± 0.2	1.4 ± 0.2	1.2 ± 0.1	0.6 ± 0.1
20	2.2	16.6 ± 0.2	18 ± 2.4	11.5 ± 1.1	16.9 ± 0.5	0.7 ± 0.1	0.4 ± 0.2	0.3 ± 0	0.1 ± 0
10	1.1	7.8 ± 3.3	10.4 ± 1.8	4.8 ± 0.9	8.8 ± 0.3	0.2 ± 0	0.4 ± 0.1	0.1 ± 0	0.1 ± 0

*n = 3

8.5 Discussion

The metals Fe, Zn, Cu and Ga were chosen based on the most likely metals present in higher concentrations in eluates from the SnO_2-based generator and their ability to influence the labelling process. The chosen range of the concentrations of metals introduced ("spiking") was based on likely concentrations of metal ions that may be present in a radiolabelling setup (10 – 20 µg) to an exaggerated quantity (250 µg) that exceeded the pharmacopoeia limits for metal contaminants in generator ^{68}Ga eluates. De Blois et al. (2011) found the total metal ions (Ga, Ge, Zn, Ti, Sn, Fe, Al and Cu) in the eluate from the SnO_2-based generator to consistently be < 10 ppm (with Zn < 3 ppm and for all the other metals < 1ppm). The 250 µg was included to assess the ability of the Bond Elut SCX (100 mg) resin to remove metal ions over a wide concentration range.

From previous studies (Oehlke et al., 2013) it was found that Fe^{3+}, Cu^{2+} and Zn^{2+} were among the metal ions found to have the largest influence on radiolabelling. They also found that complete labelling was always possible when the metal/ligand ratio was below 1:1. From the results obtained above and based on 35 µg of DOTATATE used in the labelling process, Fe was the only metal ion in **Fr2** that exceeded this metal to ligand ratio (1.69:1), when metal spiked ^{68}Ga eluates, containing 250 µg of each metal, were added to the SCX cartridge. The addition of metal spiked ^{68}Ga eluates, containing 250 µg of each metal to the SCX cartridge, resulted in 0.78:1 Cu and 0.86:1 Zn in **Fr2** (similarly based on 35 µg or 24.3 nmol of DOTATATE used in the labelling process). The addition of metal spiked ^{68}Ga eluates, containing 20 µg of each metal, to the SCX cartridge, resulted in the following metal ion: ligand ratio in **Fr2**: 0.53:1 Fe, 0.21:1 Cu and 0.25:1 Zn (based on 35 µg or 24.3 nmol of DOTATATE used in the labelling process). The addition of metal spiked ^{68}Ga eluates, containing 10 µg of each metal, to the SCX cartridge, resulted in the following metal ion: ligand ratio in **Fr2**: 0.16:1 Fe, 0.08:1 Cu and 0.25:1 Zn (based on 35 µg or 24.3 nmol of DOTATATE used in the labelling process). The ratio of Cu:ligand in **Fr2** was consistently lowest for all three (10, 20 and 250 µg) metal-spiked eluates and Fe:ligand was the highest for the two higher metal-spiked eluates (20 and 250 µg). For the lower metal-spiked eluates (10 and 20 µg), the Zn:ligand ratio in **Fr2** was consistently 0.25:1. This implies that under the conditions of this investigation, when the ^{68}Ga eluates were spiked with 10 – 20 µg of each metal, the SCX resin efficiently removed the metals to achieve a metal/ligand ratio of less than 1:1 for ideal labelling conditions (Oehlke et al., 2013). Even when unrealistically high quantities of metals of up to 250 µg were introduced, only the Fe:ligand ratio exceeded this ratio.

8.6 Conclusion

This investigation showed that, of the initial quantities (10, 20 and 250 µg) of introduced Fe, Zn, Cu and Ga in the ^{68}Ga eluate, only about 2 % of these total metals remained in the purified ^{68}Ga fraction after passing the "spiked" eluate through a Bond Elut SCX (100 mg) cartridge. It is highly unlikely that ^{68}Ga eluates would contain as much as 250 µg of any metal if sufficient care is taken to avoid the introduction of metals during the labelling process (e.g. avoiding the use of metal needles and using only analytical grade chemicals). The levels of metals, particularly the most influential Fe, Zn and Cu, were sufficiently low when the ^{68}Ga eluates were contaminated with less than 20 µg of each metal. The metal/ligand ratio for all the metals was less than 1:1, a condition that would result excellent labelling of DOTA-peptides.

Chapter 9: Optimization of radiolabelling method of ^{68}Ga-labelled DOTATATE, using SCX-processed ^{68}Ga eluates

9.1 Introduction

An optimized radiolabelling method using fractionated eluates from a SnO_2-based ^{68}Ge/^{68}Ga generator was described in Chapter 5. All the parameters that were developed during that study (e.g. buffer content of labelling mixture) would not necessarily apply when using ^{68}Ga eluates that were processed differently. The pH of the labelling mixture would be affected by the different HCl content of differently processed eluates. A radiolabelling method of DOTA-peptides was described in Mueller et al. (2012), using ^{68}Ga that had been purified on a silica-based strong cation exchange resin (SCX cartridge). The method involved the use of ammonium acetate buffer and the labelling reaction was conducted in a relatively large volume of approximately 3.7 – 3.9 ml. No post-labelling purification was conducted.

The composition of the purified eluate described in Chapter 7 of this work was similar to that of the Mueller et al. (2012) method. It was, however, decided not to copy the Mueller labelling method exactly, but rather to develop a method based on the labelling conditions described earlier in Chapter 5. This method used a sodium acetate (instead of ammonium acetate) buffer system, smaller reaction volumes and different DOTA-peptide content. Sodium acetate was used as a buffer because it ensured a radiolabelling mixture with a consistent pH throughout the labelling process and it has been successfully used in the work described in chapters 5 and 6 as well as in other well-published DOTA-labelling studies (Hoffman et al., 2001; Henze et al., 2001; Breeman et al., 2005; de Blois et al., 2011; Rossouw and Breeman, 2012; Decristoforo et al., 2007; Mukherjee et al., 2014a, 2014c; Das et al., 2014). There was no further optimization of the DOTA-peptide content, as that had already been done in Chapter 5. Instead, this systematic approach was rather to develop an optimum pH range for labelling, with the aim to improve radiolabelling yields and to reduce losses of activity during post-labelling purification.

9.2 Aim

The main aim was to optimize the labelling method using pre-concentrated ^{68}Ga eluates from a Bond Elut SCX (100 mg) cation exchange resin. Labelling conditions, based on the acidity of the concentrated eluate, and therefore the pH of the labelling mixtures, were investigated. For this purpose, only DOTATATE was selected as a source of DOTA-peptide because

previous results in this study showed that the labelling results obtained from the three DOTA-peptides (DOTATATE, DOTATOC and DOTANOC) were quite similar.

9.3 Materials and Methods

General methods are summarised in chapter 3. This section describes variations and details specific to the work described in this chapter.

9.3.1 Elution of the generator

The ^{68}Ga was eluted from an 1110 MBq (30 mCi) SnO_2-based ^{68}Ge/^{68}Ga generator with 12 ml 0.6 M HCl (see chapter 3) and collected in a borosilicate glass vial. The 12 ml eluate was divided into three 4 ml aliquots, which were each allocated for the purification on an SCX resin, followed by labelling of DOTATATE. The activities in these aliquots ranged from 141 MBq to 185 MBq depending on the age of the generator and the decay of the ^{68}Ga eluate. ^{68}Ga activities were measured in a dose calibrator.

In addition, elution was carried out in the following way to assess whether more concentrated, higher activity eluates would yield the same labelling results as the lower activity eluates: 4.0 ml was first eluted and collected in a vial, followed by a second fraction of 6 ml which was discarded. The first 4ml fraction (activities ranging from 396 MBq to 415 MBq) was allocated for SCX resin purification followed by higher activity labelling of the peptide ligand.

9.3.2 Dilution of the ^{68}Ga eluate for SCX processing

The 4 ml 0.6 M ^{68}Ga eluate fraction or aliquot (see 9.3.1) was diluted with 4 ml ultrapure pharmaceutical grade water and mixed by vortex to render a concentration of 0.3 M HCl. The activity was measured in a dose calibrator.

9.3.3 Concentration of ^{68}Ga eluates on the Bond Elut SCX cation exchange resin

See 3.4.10 for pre-conditioning and concentration of ^{68}Ga eluates on the Bond Elut SCX cation exchange resin. In the concentration of the ^{68}Ga eluates, the diluted ^{68}Ga eluate (see 9.3.2 above) was loaded onto the pre-conditioned Bond Elut SCX (100 mg) cartridge. The concentrated ^{68}Ga fraction **Fr2** (0.5 ml) was obtained as described in 3.4.10.

9.3.4 Radiolabelling of the DOTATATE with concentrated eluates and post-purification thereof

DOTATATE stock solution (1 mg/ml, 35 µl) was added to the purified ^{68}Ga solution **Fr2** (0.5 ml). Various volumes of 2.5 M NaOAc, ranging from 40 – 60 µl, were added to the ^{68}Ga DOTATATE mixture. After addition of 2 ml ultrapure pharmaceutical grade water, the contents of the vial were gently mixed and the pH was measured. Under these conditions the DOTATATE molar concentration was 9.4 – 9.5 µM. The vial was then sealed with a rubber stopper and an aluminium cap. The mixture was homogenised for 5 seconds by means of gentle swirling in a vortex mixer and the vial containing the labelling mixture was heated for 15 minutes in a controlled heating block at 95 °C. The radiolabelled DOTA-peptide reaction mixture was removed from the heating block and homogenised again by means of gentle swirling. An aliquot was removed for HPLC testing before the reaction mixture was transferred onto a purification unit consisting of a 500 mg C18, 3 cc, Sep-Pak cartridge. See 3.4.2 for the post-radiolabelling purification process.

9.3.5 Quality evaluation

9.3.5.1 pH of the concentrated eluates

The pH value of the concentrated ^{68}Ga eluate and 2.5 M NaOAc mixture, diluted in 2 ml ultrapure pharmaceutical grade water, was determined using pH indicator strips (Merck, Darmstadt, Germany). A Horiba pH meter was also used to verify the pH obtained with the pH strips and the results were consistent.

The quality of the ^{68}Ga-labelled DOTATATE was evaluated as described below.

9.3.5.2 Radiolabelling efficiency and radiochemical yield

Radiolabelling efficiency or labelling efficiency (LE) was obtained from the HPLC analysis of an un-purified labelling mixture. Radiochemical purities of ^{68}Ga-labelled DOTA-peptides before and after Sep-Pak purification were determined by means of HPLC as described in chapter 3.

For radiochemical yield, refer to 3.4.2. The activities in the collected fractions (**S1** and **S2**), as well as the remaining activities in the reaction vial and on the Sep-Pak C18 cartridge were measured in a dose calibrator.

9.4 Results

9.4.1 Determination of the optimal volume of 2.5 M NaOAc (pH) on radiolabelling results

The results in Table 9.1 present data on the influence of the volume of sodium acetate (controlling the pH of labelling mixtures) on radiolabelling and Sep-Pak C18 purification results (radiochemical yields) of [^{68}Ga]DOTATATE, using 35 µg DOTATATE and SCX-processed ^{68}Ga eluates.

Table 9.1: Influence of volume of sodium acetate on radiolabelling results of [^{68}Ga]DOTATATE, using SCX-processed ^{68}Ga eluates

Influence of volume of sodium acetate (pH) on radiolabelling and Sep-Pak C18 purification results of [^{68}Ga]DOTATATE, using 35 µg DOTATATE and SCX-processed ^{68}Ga eluates showing mean values of decay-corrected activities in various fractions, expressed as percentages of the starting activity (141 – 185 MBq or higher activity concentrations 396 – 415 MBq**), number of experiments given within brackets; S1 = first Sep-Pak fraction collected with 8 ml saline; S2 (Radiochemical Yield) = second Sep-Pak fraction collected with 0.7 ml ethanol:saline [50:50] + 2.25 ml saline; S3 = remaining activity in vial; and S4 = remaining activity in Sep-Pak after collecting S2

Vol. 2.5 M NaOAc (µl)	pH range of labelling mixture	*Labelling efficiency (%)	S1 (%)	S2 (%)	S3 (%)	S4 (%)
40 (n=4)	2.0 – 2.5	93 ± 6	6 ± 3	82 ± 4	2 ± 0.5	11 ± 2
42 (n=4)	2.5 – 3.0	96 ± 4	3 ± 2	83 ± 2	2 ± 0	12 ± 2
44 (n=4)	2.5 – 3.0	98 ± 1	2 ± 0	83 ± 3	3 ± 1	12 ± 3
46 (n=12)	3.0	99 ± 1	2 ± 0.4	84 ± 3	7 ± 4	7 ± 4
**46 (n=4)	3.0	97 ± 2	3 ± 0.5	81 ± 2	5 ± 1	12 ± 3
48 (n=4)	3.0	98 ± 0.5	3 ± 1	82 ± 2	8 ± 2	7 ± 3
50 (n=3)	3.5	99 ± 1	3 ± 1	64 ± 6	24 ± 4	9 ± 1
55 (n=1)	4.0	79	10	24	55	12
60 (n=1)	4.5	70	12	20	54	14

*Determined by means of HPLC

The addition of 40 – 50 µl NaOAc (labelling mixture pH 2.0 – 3.5) resulted in a consistent increase in the average radiolabelling efficiency of 93 % to 99 % but a significant decrease of 79 % to 70 % when volumes of 55 – 60 µl (pH 4.0 – 4.5) of NaOAc were added. The average radiochemical yield (**S2**) remained fairly constant (81 – 84 %) with the addition of 40 – 48 µl NaOAc but decreased significantly (64 – 20 %) when volumes of 50 – 60 µl (pH 3.5 – 4.5) NaOAc were added. The average activity remaining on the vial was 2 – 8 % for the addition

of 40 – 48 µl (pH 2.0 – 3.0) NaOAc, but increased dramatically thereafter with as much as around 55 % when volumes of 55 – 60 µl (pH 4.0 – 4.5) of NaOAc were added.

9.4.2 Radiolabelling efficiency and radiochemical yield results under optimized conditions

The results in Table 9.2 present data on individual radiolabelling efficiency and Sep-Pak C18 purification results (radiochemical yields) of [^{68}Ga]DOTATATE, using SCX-processed ^{68}Ga (^{68}Ga generator eluate in 0.6 M HCl, diluted to 0.3 M HCl and processed on SCX).

The radiochemical purity post Sep-Pak C18 purification was always in the order of 100 % (not tabulated). The radiolabelling efficiency results ranged from 95 – 99 % and the radiochemical yields (**S2**) ranged from 83 – 89 %. The remaining activity on vial (**S3**) was fairly consistent between 5 – 6 % and the activity remaining on the Sep-Pak cartridge (**S4**) was 3 – 10 %.

Table 9.2: Individual radiolabelling results of [^{68}Ga]DOTATATE, using SCX-processed ^{68}Ga eluates

Individual radiolabelling efficiency and Sep-Pak C18 purification results of [^{68}Ga]DOTATATE, using SCX-processed ^{68}Ga activity (^{68}Ga generator eluate in 0.6 M HCl, diluted to 0.3 M HCl and processed on SCX), labelling conducted at pH = 3 (46 µl NaOAc), using 35 µg DOTA-peptide, showing values of decay-corrected activities in various fractions, expressed as percentages of the starting activity; S1 = first Sep-Pak fraction collected with 8 ml saline; S2 (Radiochemical Yield) = second Sep-Pak fraction collected with 0.7 ml ethanol:saline [50:50] + 2.25 ml saline; S3 = remaining activity in vial; and S4 = remaining activity in Sep-Pak after collecting S2

Labelling efficiency (HPLC) (%)	S1 (%)	S2 (%)	S3 (%)	S4 (%)
99	2	83	5	10
95	3	84	6	7
99	2	89	6	3

9.5 Discussion

The results in Table 9.1 summarize a systematic investigation of the influence of pH (determined by the volume of 2.5 M NaOAc added) on the labelling yields as well as adsorption of activity on the reaction vial (**S3**).

The radiolabelling of DOTA peptides is highly pH dependent (Velikyan, et al., 2004; Breeman, et al., 2005). Breeman et al. (2005) found that pH 3.5 – 4.0 was the optimal pH for incorporation of Ga^{3+} in the labelling process. Velikyan, et al. (2010 and 2012) performed

labelling of DOTA-peptides at a pH of about 4.6. Meyer et al. (2003a) also found the optimal pH range for the radiolabelling to be 4.3 – 5.0. However, in the current study, at pH 3.5, although the radiolabelling efficiency was 99 %, the radiochemical yield (**S2**) started dropping below 65 % with almost 25 % of the activity remaining on the vial. At pH range of 4.0 – 4.5, the radiolabelling efficiency dropped below 80 %, the radiochemical yield dropped to below 25 % and as much as 55 % of the activity remained on the glass vial. This may be due to the less acidic conditions that may result in hydrolysis of the ^{68}Ga. These discrepancies between labelling efficiencies and actual radiochemical yields clearly illustrate that under certain non-optimal labelling conditions, labelling efficiency on its own could be a misleading measurement and be not a true reflection of actual radiochemical yield.

The optimal pH for the radiolabelling process in this study was found to be 3.0 based on the radiolabelling results (radiolabelling efficiency and radiochemical purity). In Table 9.2 (where individual radiolabelling efficiency and Sep-Pak C18 purification results at pH 3 after addition of 46 µl 2.5 M NaOAc, are given), the average activity remaining on the vial (**S3**) under optimized labelling conditions was 6 ± 0.6 % compared to 16 ± 4 % (for DOTATATE radiolabelling), in Chapter 5 (see Table 5.3). This may be due to the pH of the radiolabelling process having been too high (3.5 – 4.0) in the previous study.

The radiolabelling conditions given in Section 9.3.4 with regards to the molar concentration of DOTATATE (9.4 – 9.5 µM) also concur with the optimum conditions established in Chapter 5 (9.2 – 9.4 µM).

It was found that both elution methods (using a concentrated or less concentrated 4 ml ^{68}Ga eluate, see 9.3.1) resulted in similar labelling results (see Table 9.1, entry 5**) and hence the first elution method was predominantly used to increase the number of studies that could be performed with a single elution. When higher activity is required, however, eluates with higher activity concentration can be introduced into labelling mixtures (without altering the eluate volume).

9.6 Conclusion

The successful development of a labelling method using pre-concentrated ^{68}Ga eluates from a Bond Elut SCX (100 mg) cation exchange resin was achieved. The method produced a high radiolabelling efficiency yield of around 99 % and high radiochemical yield of almost 85 %. The labelling results for DOTA-peptide labelling at a pH of 3 were found to be superior to those at a higher pH (3.5 – 4.0). It should be possible to adapt this method to an automated synthesis module and the procedure makes ^{68}Ga-labelled products accessible with the advantage (over the acetone-based methods) of reducing the preparation time due to not having to remove organic solvents in the process, and avoid testing for acetone in the final product, which reduces the costs in the radiopharmacy or clinical setup.

Chapter 10: Development and evaluation of user-friendly single vial DOTATATE kit formulations, using SCX-processed ^{68}Ga eluates

10.1 Introduction

The cation exchange-based post-processing method described in Chapter 7 reduces ^{68}Ga eluate volumes and removes most chemical contaminants and ^{68}Ge breakthrough. In order to improve the user-friendliness of the radiolabelling method of DOTA-peptides, using SCX-processed ^{68}Ga eluates obtained from a SnO_2-based generator (described in Chapter 9), there is a need to develop DOTA-peptide kit formulations based on this optimised radiolabelling method.

In most previously published reports, DOTA-peptide kit formulations for labelling were based on the use of ^{68}Ge/^{68}Ga generators eluted with relatively low HCl concentrations. In both the studies by Mukherjee et al. (Mukherjee et al., 2014a, 2014c), the ^{68}Ga eluates (in 0.1 M HCl) from a nanoceria-polyacrylonitrile (PAN), composite-sorbent-based ^{68}Ge/^{68}Ga generator were directly added to the kit formulation without any pre- or post-processing methods. In another study by the same author (Mukherjee et al., 2014b) DOTATATE kits for radiolabelling with both ^{68}Ga and ^{177}Lu were formulated as a theranostic radiopharmaceutical preparation. The ^{68}Ga was eluted from a SnO_2 generator with 0.1 M HCl, which was loaded on a Strata-X-C cartridge and the purified ^{68}Ga was eluted in an acetone/HCl solution and directly added to the kit vial. Das et al. (2014) conducted their study using a TiO_2-based generator and freeze-dried peptide kits containing equal quantities of DOTATATE and DOTANOC. The ^{68}Ga eluates were purified on a Strata-X-C cation exchange cartridge using HCl/acetone solution and further purification of the ^{68}Ga-labelled peptides was carried out on a Sep-Pak C18 reverse phase cartridge. A study by Asti et al. (2015) to develop and optimise direct labelling of DOTATOC through a kit-based approach was conducted using fractionated eluates from a TiO_2-. and a silica-based generator.

The advantages of using kit-type formulations for the radiolabelling of DOTA-peptides with ^{68}Ga have been earlier described in Chapter 6. Those formulations, however, were developed for use with fractionated eluates. This investigation describes the development of single vial DOTATATE kits, formulated with sodium acetate as a buffer and to be used with SCX-processed ^{68}Ga eluates, using a SnO_2-based ^{68}Ge/^{68}Ga generator as the source of ^{68}Ga. It includes the monitoring of radiochemical yields of the ^{68}Ga-labelled DOTATATE,

radiolabelling efficiency and other quality control parameters (pH, sterility and apyrogenicity) over a 3-month period.

10.2 Aim

After optimized labelling conditions were established by using SCX-processed ^{68}Ga eluates (see Chapter 9), the following aim was to investigate the development of single vial DOTATATE kit formulations and to test their radiolabelling efficiency using the processed eluates as the source of ^{68}Ga. As was the case in Chapter 6, the primary objective of this investigation was to improve the convenience and user-friendliness of the labelling procedure. The next step was to evaluate the quality of the kits over a period of at least 3 months in order to establish the stability over this period when kept frozen at -20 °C. The efficiency of the kits (prepared using four different methods) was also investigated. Additionally, an alternative quality control method for the un-purified labelled DOTATATE was used to determine the possible presence of colloids, which are un-detectable by HPLC. This would provide a guideline whether C18 post-labelling purification is required when labelling is done in conjunction with the SCX eluate processing method.

10.3 Materials and methods

General methods are summarised in chapter 3. This section describes variations and details specific to the work described in this chapter.

10.3.1 Elution of the generator

The ^{68}Ga was eluted from an 1110 MBq (30 mCi) SnO_2-based ^{68}Ge/^{68}Ga generator with 12 ml 0.6 M HCl (see 3.4.1) and collected in a borosilicate glass vial. The 12 ml eluate was divided into three 4 ml aliquots, which were each purified on the (Bond Eut SCX) cation exchange resin and eventually used for labelling of DOTATATE. The activities in these aliquots ranged from 63 MBq to 185 MBq depending on the age of the generator and the decay of the ^{68}Ga eluate. ^{68}Ga activities were measured in a dose calibrator.

10.3.2 Preparation of DOTATATE single vial kits

DOTATATE stock solution (1 mg/ml) was prepared using ultra-pure pharmaceutical grade water and stored at -20 °C (see 3.3.2). The solution of 2.5 M NaOAc was made with ultra-pure pharmaceutical grade water and filtered through a 22-micron sterile filter. Single vial kits were prepared using the following four different methods:

10.3.2.1 DOTATATE Single Vial Kits A

A pre-calculated mass of 15.64 mg of sodium acetate trihydrate salt was weighed into individual sterile vials and 35 µl of DOTATATE stock solution was added into each vial. The unsealed single vial kits were individually vacuum-dried under sterile conditions in a desiccator connected with a N 726 FT.18 vacuum pump (KNF Neuberger Inc., Trenton, USA) for 3 hours at room temperature, the vials were then sealed with rubber stoppers and aluminium caps, and stored at -20 °C until use.

10.3.2.2 DOTATATE Single Vial Kits B

A volume of 46 µl of 2.5 M NaOAc was pipetted into each sterile labeled vial and 35 µl of thawed DOTATATE stock solution (containing 35 µg peptide) was added to each of these vials. The unsealed single vial kits were individually vacuum-dried under sterile conditions in a desiccator connected with a vacuum pump for 3 hours at room temperature, the vials were then sealed with rubber stoppers and aluminium caps, and stored at -20 °C until use.

10.3.2.3 DOTATATE Single Vial Kits C

A volume of 46 µl of 2.5 M NaOAc was pipetted into each sterile labeled vial and 35 µl of thawed DOTATATE stock solution (containing 35 µg peptide) was added to each of these vials. No vacuum drying was applied. The single vial kits were sealed with rubber stoppers and aluminium caps. The contents of the vials were mixed with a vortex action and the vials stored at -20 °C until use.

10.3.2.4 DOTATATE Single Vial Kits D (freeze dried)

A volume of 46 µl of 2.5M NaOAc was pipetted into each sterile labeled vial and 35 µl of thawed DOTATATE stock solution (containing 35 µg peptide) was added to each of these vials. These single vial kits were frozen with liquid nitrogen and loaded into the freeze dryer with a shelf temperature of -50 ºC for 4 h. The shelf temperature was increased to 35 ºC to sublime the ice. Each vial was vacuum-sealed, weighed and stored in a freezer at -20 °C until use.

The frozen single vial kits were stored in a freezer at -20 °C up to 3 months and removed from the freezer and brought to room temperature before being used in each labelling process.

10.3.3 Concentration of ^{68}Ga eluates on the SCX cation exchange resin

See 3.4.10 for pre-conditioning and concentration of ^{68}Ga eluates on the Bond Elut SCX (100 mg) cation exchange cartridge. The concentrated fraction **Fr2** (0.5 ml) was eluted directly from the SCX resin into a sterile kit vial (containing the DOTATATE and 46 µl 2.5 M NaOAc or 15.64 mg sodium acetate trihydrate) instead of an empty vial.

10.3.4 Radiolabelling of the DOTATATE single vial kits and post-purification process

Ultrapure pharmaceutical grade water (2 ml) was added to the 10 ml vial containing purified ^{68}Ga and the kit, the vial was gently mixed and the pH was measured. Under these conditions the DOTATATE molar concentration was 9.7 µM. The vial was then sealed with a sterile rubber stopper and an aluminium cap. The mixture was homogenised for 5 seconds by means of gentle swirling in a vortex mixer and the vial containing the labelling mixture was heated for 15 minutes in a controlled heating block at 95 °C. The radiolabelled DOTA-peptide reaction mixture was removed from the heating block and homogenised again by means of gentle swirling. An aliquot was removed for HPLC and iTLC testing before the reaction mixture was transferred onto a purification unit consisting of a 500 mg C18, 3 cc, Sep-Pak cartridge. See 3.4.2 for the post-radiolabelling purification process.

10.3.5 Quality Evaluation of the DOTA-peptide kits

The quality and stability of the DOTATATE single vial kits (A, B, C and D) were evaluated at monthly intervals over a 3-month period as described below.

10.3.5.1 Radiolabelling efficiency and radiochemical yield

Radiolabelling efficiency or labelling efficiency (LE) of DOTATATE, using single vial kits and ^{68}Ga eluates that had been processed on the Bond Elut SCX resin, was determined by HPLC analysis (see 3.4.3), as well as by an iTLC method in order to detect for possible colloids (see below). Radiochemical purities of Sep-Pak C18-purified ^{68}Ga-labelled DOTA-peptides were also determined by means of HPLC.

For radiochemical yield, refer to 3.4.2. The activities in the collected fractions (**S1** and **S2**), as well as the remaining activities in the reaction vial and on the Sep-Pak C18 cartridge were measured in a Dose Calibrator.

Radiolabelling efficiency, radiochemical purity and radiochemical yields were monitored over a 3-month period.

10.3.5.2 Colloid determination of the pre-Sep-Pak un-purified ^{68}Ga-labelled DOTA-peptides, using an iTLC method

The method of Mukherjee et al. (2014c) was used. 1 M ammonium acetate:methanol (1:1) mobile phase for iTLC was prepared as follows: A mass of 77.08 g of ammonium acetate (Sigma-Aldrich, catalogue number 431311) was weighed out and diluted to 1 litre with ultra-pure water. Equal volumes of 1 M ammonium acetate solution and methanol (catalogue number 34860 from Sigma-Aldrich) were added together immediately before use to make a 1:1 ammonium acetate:methanol solution.

Radiolabelling efficiency was determined as described below:
The iTLC-SG strips were prepared as follows: 9 cm long, 1.5 cm wide and the spot with activity (approximately 0.37 MBq) was placed at 1.5 cm from the bottom of the strip. The iTLC strip was then inserted in a chromatography tank containing 10 ml of the mobile phase and allowed to develop. Development time was approximately 10 minutes. The strip was removed from the tank and allowed to air dry before it was scanned using an Omni-rad EZ Scan (Carroll & Ramsey Associates, Berkeley, CA, United States), coupled to a Chromatopac C-R8A integrator (Shimadzu, Kyoto, Japan). The chromatograms displayed peaks with retention times in minutes (R_t), which were converted to the retention factor (Rf) values using the method of Davids (2017). The radiolabelling efficiency (iTLC) was obtained directly from the integrated values on the iTLC chromatogram. It was expressed as the amount of labelled DOTA-peptide with R_f value = 0.9, expressed as a percentage of the total amount of radioactivity species recorded on the iTLC radio-chromatogram. See Annex 1 for an example of the chromatogram.

10.3.5.3 Microbiological evaluation of DOTATATE cold kits

Sterility testing on the formulated DOTATATE cold kits was performed according to the methods prescribed in the European Pharmacopoeia (2013). A total of twelve samples of the DOTATATE single vial kits (3 of each kit type) were tested. These DOTATATE single vial kits were each dissolved (up to 0.5 ml) in a mixture of 5 M NaCl and 5.5 M HCl 40:1 (v/v) and then analysed for bacterial growth using soybean-casein digest medium for aerobic bacteria and fungi, and fluid thioglycollate medium for anaerobic bacteria. The sterile 5 M

NaCl and 5.5 M HCl 40:1 (v/v) was used to closely represent the ^{68}Ga eluate that would be added to the kit but avoid false negative results that may occur due to the presence of the ^{68}Ga. The 5 M NaCl and 5.5 M HCl was prepared using ultrapure pharmaceutical grade water and filtered (through a 0.22-micron filter) before use. See Chapter 3 for sterility testing method.

Endotoxin testing was performed on a total of twelve DOTATATE cold single vial kits (3 of each kit type) dissolved in sterile saline. The testing was performed according to the European Pharmacopoeia (2013) using the chromogenic method as described in Chapter 3. The calibration curve was used to determine the concentration of endotoxins in the DOTATATE kits.

10.3.5.4 pH

The pH value of the radiolabelled DOTA-peptide reaction mixture was determined using pH indicator strips (Merck, Darmstadt, Germany).

10.3.5.5 Testing for ^{68}Ge in radiolabelled preparations

The same method described in *3.4.6* was used.

10.4 Results

10.4.1 Determination of kit composition parameters and preparation of single vial kits

Based on the results in Chapter 5, where fractionated ^{68}Ga eluates were used, 35 µg of DOTA-peptide was used in the formulation of the kits and for all the radiolabelling experiments. The results in Chapter 9, emanating from the use of SCX-processed ^{68}Ga eluates, were used to calculate the required quantity of NaOAc for the kits. The inclusion of 15.64 mg of sodium acetate trihydrate salt (in Kits A) or 46µl of 2.5M NaOAc (in Kits B, C and D) as buffer in the kit formulation ensured a radiolabelling mixture with a consistent pH of 3.0.

10.4.2 Quality Evaluation of the DOTA-peptide kits

10.4.2.1 Radiolabelling efficiency (HPLC) and radiochemical yield

The results in Table 10.1 present data on the radiolabelling efficiency of [^{68}Ga]DOTATATE, using SCX-processed ^{68}Ga eluates and single vial kits containing 35 µg DOTATATE, stored for various periods at -20 ˚C.

Over the 12-week storage period, radiolabelling efficiency results obtained from all four the DOTATATE single vial kit types were consistently above 98 %, irrespective of the type of

kit used. The average radiolabelling efficiency of ^{68}Ga-labelled DOTATATE obtained from the DOTATATE single vial kits A at the end of the 12-week storage period was 98.3 %, for DOTATATE single vial kits B it was 98.7 %, for DOTATATE single vial kits D it was 99.0 % and it was the highest for DOTATATE single vial kits C, at 99.7 %.

Table 10.1: HPLC pre-Sep-Pak purification radiolabelling efficiency results of [^{68}Ga]DOTATATE, using SCX-processed ^{68}Ga eluates and single vial kits

Peptide Storage Period	Radiolabelling efficiency (%)			
	Kit A	Kit B	Kit C	Kit D
1 week (n=3)	99.7 ± 1	99.0 ± 1	99.0 ± 1	-
4 weeks (n=3)	99.3 ± 1	99.0 ± 1	99.0 ± 2	99.0 ± 0
8 weeks (n=3)	99.3 ± 1	98.7 ± 2	99.3 ± 1	99.0 ± 0
12 weeks (n=3)	98.3 ± 1	98.7 ± 2	99.7 ± 1	99.0 ± 1

*For kit preparation, see 10.3.2

Over the 12-week storage period, Sep-Pak C18 radiochemical yields obtained with the single vial DOTATATE single vial kit types ranged from 75 – 89 % (see Table 10.2). No obvious decline in the radiochemical yields was observed over a 12-week kit storage period. The average radiochemical yield obtained from the DOTATATE single vial kits A after a 12-week storage period was 80 % compared to 84 % for the DOTATATE single vial kits B and C. The average radiochemical yield obtained from the DOTATATE single vial kit D was the highest (87 %) after the same storage period.

The radiochemical purity post Sep-Pak C18 purification was always in the order of 100 % (not tabulated).

Table 10.2: Sep-Pak C18 radiochemical yields of [^{68}Ga]DOTATATE, using single vial kits containing 35 µg DOTATATE, prepared using four different methods and stored for various periods at -20 ˚C

Kit Storage Period at -20 ˚C	Radiochemical Yield (%)			
	Kit A	**Kit B**	**Kit C**	**Kit D**
1 week (n=3)	82 ± 2	77 ± 2	82 ± 3	-
4 weeks (n=3)	78 ± 7	75 ± 11	86 ± 1	75 ± 7
8 weeks (n=3)	79 ± 4	82 ± 4	89 ± 5	77 ± 2
12 weeks (n=3)	80 ± 6	84 ± 5	84 ± 4	87 ± 1

*For kit preparation, see 10.3.2

10.4.2.2 Colloid determination of the pre-SEP PAK un-purified [^{68}Ga]DOTATATE, using an iTLC method

The R_f results of Mukherjee et al. (2014c), whose iTLC method was used in this investigation, served as a guideline to identify the location of the various radioactive species on the developed iTLC chromatogram. The R_f-value of the labelled peptide was given as 0.9 – 1, while the R_f of free ^{68}Ga as well as colloidal ^{68}Ga was given as 0 – 0.1. Under those iTLC conditions, no detectable quantities of colloids were present in the un-purified ^{68}Ga-labelled DOTATATE, as indicated by the iTLC analysis results presented in Table 10.3. Over the 12-week storage period, radiolabelling efficiency (iTLC) results on pre-purified ^{68}Ga-labelled DOTATATE, using the DOTATATE single vial kit types and labelled with SCX-processed ^{68}Ga, were consistently above 99 %, with the exception of DOTATATE single vial kits D which was only slightly less (98.7 %). The average radiolabelling efficiency (iTLC) of ^{68}Ga-labelled DOTATATE obtained from the DOTATATE single vial kits A at the end of the 12-week storage period was 99.5 %, for DOTATATE single vial kits B and C was 100 %, and was the lowest for DOTATATE single vial kits D, at 98.7 %.

Radiochemical purities of Sep-Pak C18-purified ^{68}Ga-labelled DOTA-peptides were also determined by means of HPLC.

10.4.2.3 Microbiological evaluation of DOTATATE cold kits

All the samples were found to be clear and sterile with no aerobic bacterial, anaerobic bacterial or fungal growth. The average endotoxin content of the radiolabelled DOTATATE single vial Kits C was the lowest at 1.1 IU/ml (0.97 – 1.39 IU/ml), followed by 1.4 IU/ml for Kits B (1.27 – 1.50 IU/ml), then 1.6 IU/ml for Kits D (1.57 – 1.68 IU/ml) and highest for Kits A at 1.8 IU/ml (1.70 – 2.0 IU/ml) (see Table 10.3). The European Pharmacopoeia (2013) specification for bacterial endotoxin is less than 175 IU/V, where V is the maximum volume to be used for the preparation of a single patient dose, if intended for use in the manufacture of parenteral preparations without a further procedure for the removal of bacterial endotoxins. The maximum volume of the pre-purified solution (**Fr2** and kit) is 0.581 ml and the total volume of the solution (**S2**) post-purification is 3.55 ml.

10.4.2.4 pH

The pH values of the DOTATATE radiolabelling solutions (for Kits A, B, C and D) were all consistently 3.0 over the 3-month period.

A summary of the quality control results between DOTATATE single vial kits A, B, C and D after a storage period of 12 weeks at -20 °C is given in Table 10.3.

Table 10.3: Radiolabelling and microbiological quality control results, using SCX-processed ^{68}Ga eluates and single vial kits containing 35 µg DOTATATE, after storage for 3 months at -20 ˚C

Quality Control Parameters	Kit A	Kit B	Kit C	Kit D
Radiolabelling Efficiency (%) (HPLC)	98.3 ± 1	98.7 ± 2	99.7 ± 1	99.0 ± 1
Radiolabelling Efficiency (%) (iTLC)	99.5 ± 1	100 ± 0	100 ± 0	98.7 ± 1
Radiochemical Yield (%) (Sep-Pak)	80 ± 6	84 ± 5	84 ± 4	87 ± 1
Activity lost in reaction vial (%)	6 ± 2	3 ± 0.6	3 ± 0.6	5 ± 1.5
Endotoxin count (of cold kit) (IU/ml)	1.8 ± 0.2	1.4 ± 0.1	1.1 ± 0.2	1.6 ± 0.1
Sterility (of cold kit)	Sterile	Sterile	Sterile	Sterile
pH	3.0	3.0	3.0	3.0

*n = 3

10.5 Discussion

Various investigators (Asti et al., 2015; Das et al., 2014; Mukherjee et al, 2014a, 2014c) developed DOTA-peptide kits that were formulated for use with ^{68}Ga eluates obtained from TiO_2- or nanoceria-PAN- based generators. These types of generators are eluted with a low concentration of HCl (0.1 M). Das et al. (2014) and Mukherjee et al. (2014b) developed DOTA-peptide kits for use with cationic resin-purified ^{68}Ga. These cationic resin purification processes involved the use of the acetone/HCl elution method. Kit freeze-drying was employed in all of these methods. Das et al. (2014) and Mukherjee et al. (2014b) performed further purification of the ^{68}Ga-labelled peptides on a C18 reverse phase Sep-Pak cartridge.

This investigation focused on the formulation and evaluation of DOTATATE kits, which were formulated specifically for radiolabelling with SCX resin-purified ^{68}Ga eluates from a SnO_2-based ^{68}Ge/^{68}Ga generator. The formulation was based on work described in Chapter 6, with some modifications. The initial investigation was performed with DOTATATE, DOTATOC and DOTANOC kits and the radiolabelling and quality results (not shown) were found to be similar. Further evaluation in this investigation was performed on the DOTATATE kits only. Based on previous other investigations it was decided that 35 µg of DOTATATE was an optimal quantity of DOTA-peptide to include in the single vial kit formulations for radiolabelling purposes (see Chapters 5 and 9). The pre-determined mass of sodium acetate trihydrate buffer (or volume of 2.5 M NaOAc) required for the DOTA-peptide single vial kits was based on the quantity of buffer required to adjust the pH of the solution to a pH of 3.0 suitable for DOTA-peptide labelling (see Chapter 9). The radiolabelling conditions given in Section 10.3.4 with regards to the molar concentration of DOTATATE (9.7 µM) concur with the optimum conditions established in Chapter 5 (9.2 – 9.4 µM).

The radiolabelling efficiency results obtained by HPLC for all four kit types, was more than 98 % over the 3-month storage period. The radiochemical yield for the ^{68}Ga-labelled DOTATATE with Kit C gave the best results (above 80 %) for the entire 3-month period. There was no decline or clear trend in the radiochemical yield for any of the kit types over the 3-month storage period. Based on the results, the different methods of kit preparation do not have a significant influence on the radiolabelling results. This implies that any one of the four methods can be used to prepare the kits in a radiopharmacy or clinical environment depending on the convenience and available infrastructure. The results in Table 10.2, however, show that the labelling yields obtained from Kit C were the most consistent over the entire 12-week period. The ultimate use of this kit preparation method might therefore be the most

advantageous, also in terms of ease and user-friendliness, as it requires no extra weighing of ingredients or drying of the kit. Freeze-drying (method D) is the most commonly used method in the literature for peptide kit preparation, but may not be possible in a clinical or radiopharmacy set-up that does not possess a freeze-dryer.

The radiolabelling efficiency results obtained from the iTLC method in Table 10.3, which was almost all above 99 %, imply the absence of colloids. No ^{68}Ge was found in any of the ^{68}Ga-labelled DOTATATE preparations due to the SCX-processing (although the results were not reported above). These two results suggest that there might be no need for the post-labelling purification of the ^{68}Ga-labelled peptide when labelling is done under these conditions. In a radiopharmacy setup, a simple iTLC analysis may be performed to detect colloids or free ^{68}Ga, before the preparation can be diluted for injection. The absence of a need for post-labelling purification would have a big advantage in terms of the length of the whole procedure and therefore the overall radiochemical yield.

The average endotoxin count for the DOTA-peptide kits was 1.1 – 1.8 IU/ml with the lowest count (1.1 IU/ml) for Kit C type and the highest (1.8 IU/ml) for Kit A. Based on the microbiological evaluation results, the radiolabelled DOTA-peptide kits were found to be sterile, pyrogen-free and microbiologically suitable for clinical intravenous application according to the European Pharmacopoeia (2013) specifications.

The pH of the DOTA-peptide kits was consistently 3.0 over the 3-month storage period, which falls within the acceptable pH range required for the DOTA-peptide labelling process. The results in Table 10.3 show that the activity remaining in the reaction vial after labelling ranged from 3 to 6 % for all the kit types. This concurs with the results obtained in Chapter 9, in which 5 to 7 % was lost, using the same quantity of buffer salt (and therefore the same pH) as in this study. The results in Chapter 5 show activity losses in vials of 16 ± 4 % (for 35 µg DOTATATE radiolabelling), while those in Chapter 6 show losses of 9 % (for kits containing 35 µg DOTATATE after 1- to 4-month storage period). The higher activity losses in the latter two studies may be due to the pH of the radiolabelling process having been too high (3.5 – 4.0) in those studies. The observation made in Chapter 6 that the lower activity lost during kit labelling may be due to slightly higher stability of the DOTA-peptide in the kit formulation might also be applicable here. This means that a combination of a lower pH and using kit- instead of non-kit-labelling may have an enhanced effect on the inhibition of activity losses, and therefore result in higher radiochemical yields.

The use of these kits reduces the labelling process time that is an advantage because due to the short half-life of ^{68}Ga, longer processing times result in losses of activity for patient use. Based on the results in Table 10.3, the stability of the single vial DOTATATE kits, allows for the preparation and storage of these user-friendly kits for a period of at least up to 3 months. Additional investigation of the DOTA-peptide kits over longer storage periods may even extend this period further. The results in Chapter 6, Table 6.2, which show that the kit performance after a period of one year was still acceptable, suggest that the kits prepared in this investigation might exhibit a similar stability.

This study is a first, according to the best of the investigator's knowledge, which presents the use of ^{68}Ga eluates from a SnO_2 generator, purified with the acidified NaCl/SCX cationic resin technology, in DOTA-peptide kit labelling.

10.6 Conclusion

The objective of formulating user-friendly stable single vial kits of DOTATATE specifically for SCX-processed ^{68}Ga eluates has been achieved. The single vial kits containing DOTATATE and sodium acetate buffer, may be prepared in 4 slightly different (fairly robust) ways. The kit manufacturing procedures described in this study are relatively simple and one of the procedures does not require a lyophilisation or vacuum-drying step. The kits can be radiolabelled with ^{68}Ga reliably and consistently in a clinical environment. Results showed that post-labelling purification might not be required, as no significant quantities of free or colloidal ^{68}Ga were detected in the labelling experiments. The preceding SCX purification step had also ensured the removal of all traces of ^{68}Ge breakthrough. The results of this kit labelling procedure therefore suggest that a post-labelling Sep-Pak C18 purification should be optional, pending validation of a reliable quality control procedure.

Chapter 11: Summary of findings and final conclusion

11.1 Summary of findings

The most significant findings relating to the SnO_2-based $^{68}Ge/^{68}Ga$ generator in this study are listed below:

An increase in the HCl concentration of the eluent resulted in increasing generator elution yields but also higher ^{68}Ge breakthrough, especially with HCl concentrations in excess of 0.6 M. The latter finding is in contrast with those of a few other investigators. Zn is the only metal tested for that shows a consistent increase with increasing HCl concentrations. This could affect labelling if eluents contain very high concentrations of HCl and eluates are not pre-purified, even taking into account that Zn^{2+} has a lower effect on labelling than metals such as Fe^{3+} and Cu^{2+}. The increased ^{68}Ge breakthrough, resulting from the use of eluents containing high concentrations of HCl, could be problematic with regards to radiation safety and radioactive waste control. Even if ^{68}Ge can be efficiently excluded from radiopharmaceutical products by means of pre-labelling purification of eluates and/or post-labelling purification of labelled products, the proper disposal of ^{68}Ge-containing waste should still be reckoned with. In order to compromise optimal elution yield with minimal ^{68}Ge breakthrough, based on the results of this study, 0.6 M HCl should therefore be the eluent of choice for a SnO_2-based generator.

For all three DOTA-peptides**,** a molar concentration of 9.2 – 9.4 µM (35 µg in 2.61 ml) was found to be the optimum to ensure radiolabelling efficiencies consistently greater than 95 % and average radiochemical yields above 70 %. Extended heating periods and the use of various heating modes offer no advantage in terms of obtaining higher radiochemical yields. Results also suggest that radiochemical yields close to 70 % can be still achieved if generators are left non-eluted for up to 7 days. Even after 21 days of non-elution of the $^{68}Ge/^{68}Ga$ generator, average radiolabelling efficiencies were above 90 % and average radiochemical yields in excess of 60 %. The purification of ^{68}Ga-DOTANOC on C18 Sep-Pak cartridges requires only a slight adjustment on the ethanol content of the eluent.

After fractional elution, the use of DOTA-peptide single vial kits offers a significant advantage in terms of convenience, stability and user-friendliness of the radiolabelling procedure. The kit manufacturing procedure described in this study is relatively simple and does not require a lyophilisation step. An added advantage is the reduced activity lost during C18 purification of the [^{68}Ga]-DOTA-peptide, resulting in slightly higher radiochemical

yields. The use of the labelling kit formulations (using the fractionated elution method) appears to be highly feasible and the quality of the DOTA-peptide kits was found to be superior over DOTA-peptide stock solutions, as reflected by the radiochemical yield results obtained after an extended kit storage period.

A cationic resin-based eluate purification method based on that developed by Mueller et al. (2012) for eluates from a TiO_2 generator, was successfully adapted for the SnO_2-based generator, which is eluted with higher concentrations of HCl, by diluting the eluate. The ^{68}Ga was efficiently adsorbed on a Bond Elut SCX (100 mg) cartridge and then desorbed by acidified solutions of NaCl. It offers the advantage over the acetone-based cationic processes of avoiding the organic solvent during the labelling process.

This investigation showed that, of the initial quantities (10, 20 and 250 µg) of introduced Fe, Zn, Cu and Ga in the ^{68}Ga eluate, only about 2 % of these total metals remained in the purified ^{68}Ga fraction after passing the "spiked" eluate through a Bond Elut SCX (100 mg) cartridge. It is highly unlikely that ^{68}Ga eluates would contain as much as 250 µg of any metal if sufficient care is taken to avoid the introduction of metals during the labelling process (e.g. avoiding the use of metal needles and using only analytical grade chemicals). The levels of metals, particularly the most influential Fe, Zn and Cu, were sufficiently low when the ^{68}Ga eluates were contaminated with less than 20 µg of each metal. The metal/ligand ratio for all the metals was less than 1:1, a condition that would result excellent labelling of DOTA-peptides.

The successful development of a labelling method using pre-concentrated ^{68}Ga eluates from a Bond Elut SCX (100 mg) cation exchange resin was achieved. The method produced a high radiolabelling efficiency yield of around 99 % and high radiochemical yield of almost 85 %. The labelling results for DOTA-peptide labelling at a pH of 3 were found to be superior to those at a higher pH (3.5 – 4.0). It should be possible to adapt this method to an automated synthesis module and the procedure makes ^{68}Ga-labelled products accessible with the advantage (over the acetone-based methods) of reducing the preparation time due to not having to remove organic solvents in the process, and avoid testing for acetone in the final product, which reduces the costs in the radiopharmacy or clinical setup.

The objective of formulating user-friendly stable single vial kits of DOTATATE specifically for SCX-processed ^{68}Ga eluates has been achieved. The single vial kits containing

DOTATATE and sodium acetate buffer, may be prepared in 4 slightly different (fairly robust) ways. The kit manufacturing procedures described in this study are relatively simple and one of the procedures does not require a lyophilisation or vacuum-drying step. The kits can be radiolabelled with ^{68}Ga reliably and consistently in a clinical environment. Results showed that post-labelling purification might not be required, as no significant quantities of free or colloidal ^{68}Ga were detected in the labelling experiments. The preceding SCX purification step had also ensured the removal of all traces of ^{68}Ge breakthrough. The results of this kit labelling procedure therefore suggest that a post-labelling Sep-Pak C18 purification should be optional, pending validation of a reliable quality control procedure.

A few limitations of this study were however identified and are listed below.

11.2 Limitations of study

- All experiments (elution, labelling and quality control) were performed manually and the number of experiments was limited, based on radiation exposure to the investigator.
- The study was performed on radiolabelling of three DOTA-peptides (DOTATATE, DOTATOC and DOTANOC) and further investigation with chelators such as 1,4,7-triacyclononane-N,N',N''-triacetic acid (NOTA) and other peptides such as Prostate-Specific Membrane Antigen (PSMA) would provide additional value.
- Pre-clinical and clinical work was not included in this study.
- Possible chemical impurities could not be identified with the HPLC equipment used.

Based on the findings, recommendations for users of this SnO_2-based generator were identified and listed below.

11.3 Recommendations

- Based on the elution yield and ^{68}Ge breakthrough, the SnO_2-based generator must be eluted with 0.6 M HCl.
- The use of single vial DOTA-peptide kits compared to DOTA-peptide stock solutions in radiolabelling is encouraged because of the reduction in processing time and improved radiolabelling results.
- Users of this generator should be aware of the ^{68}Ge breakthrough and accommodate for this in their waste disposal systems.

- The generator should preferably be eluted daily (to reduce ^{68}Ge and zinc contaminants in the eluate) and just prior to the radiolabelling process.
- Metal contamination (e.g. through the use of needles and poor quality chemicals) should be avoided as far as possible during the elution and labelling process.
- Alternative cationic resins should be further investigated by optimizing resin column sizes as well as ^{68}Ga loading and desorbing solutions from the resin.
- The cationic concentration/purification method is preferred over the fractionation method due to the reduced ^{68}Ga eluate volume and no further need for Sep-Pak C18 post-purification.
- The optimized labelling procedures and single vial kits should be developed for the use in an automated system.
- Kits for clinical application should be prepared under full cGMP conditions.
- The pH of the ^{68}Ga-labelled kits is below the European Phamacopoeia pH specification of 4.0 – 8.0 and further stability and compatibility studies are required for addition of an appropriate buffer to adjust the pH prior to patient administration.
- These kits were specifically developed for the the SnO_2-based ^{68}Ge/^{68}Ga generator and compatibility of using these kits with other ^{68}Ge/^{68}Ga generators should be explored.

11.4 Final conclusion

This work provides further insights into the application of the SnO_2-based ^{68}Ge/^{68}Ga generator as the source of the PET radionuclide ^{68}Ga, as well as the radiolabelling of somatostatin DOTA-peptide derivatives with ^{68}Ga eluted from this generator, using differently processed ^{68}Ga eluates. Investigation into the elution characteristics of the generator with various HCl concentrations indicated that elution with HCl concentrations in excess of 0.6 M is not recommended. This recommendation is based on the findings that the level of ^{68}Ge breakthrough increased significantly at concentrations of 0.8 M HCl and higher. The benefit of higher elution yields obtained at these high HCl concentrations is outweighed by the drawback of generating increasing levels of radionuclidic waste in the form of ^{68}Ge. The results of labelling studies using differently processed ^{68}Ga eluates should provide a guideline for researchers at various institutions who are engaged in the manual radiolabelling of DOTA-peptides and who are using this generator to decide which eluate processing method would be the method of their choice in their own unique clinical environment. Based on the results of this study, the cationic resin purification of ^{68}Ga eluates prior to labelling should, however, be encouraged. It has three major advantages, namely the complete removal of ^{68}Ge

contaminants, the efficient removal of unwanted metals that could interfere with labelling as well as the reduction of eluate volumes. The use of single vial DOTA-peptide kit labelling over DOTA-peptide stock solutions is advantageous largely based on the stability, user-friendliness and reduced process time which results in increased available ^{68}Ga activity (due to a shorter period allowed for decay). No Sep-Pak C18 processing is required post SCX cation processing provided there is no free ^{68}Ga and/or colloids. Users should confirm this through validation studies in their own settings.

REFERENCES

Aardaneh, K., Van der Walt, T.N., 2006. Ga_2O for target, solvent extraction for radiochemical separation and SnO_2 for the preparation of a $^{68}Ge/^{68}Ga$ generator. J Radioanal Nucl Chem. 268(1), 25-32.

Ambrosini, V., Tomassetti, P., Castellucci, P., Campana, D., Montini, G., Rubello, D., Nanni, C., Rizzello, A., Franchi, R., Fanti, S., 2008. Comparison between ^{68}Ga-DOTA-NOC and ^{18}F-DOPA PET for the detection of gastro-entero-pancreatic and lung neuroendocrine tumours. Euro. J. Nucl. Med. Mol. Imaging. 35, 1431-1438.

Ambrosini, V., Nanni, C., Zompatori, M., Campana, D., Tomassetti, P., Castellucci, P., Allegri, V., Rubello, D., Montini, G., Franchi, R., Fanti, S., 2010a. ^{68}Ga-DOTA-NOC PET/CT in comparison with CT for the detection of bone metastasis in patients with neuroendocrine tumours. Eur. J. Nucl. Med. Mol. Imaging. 37, 722-727.

Ambrosini, V., Campana, D., Bodei, L., Nanni, C., Castellucci, P., Allegri, V., Montini, G.C., Tomassetti, P., Paganelli, G., Fanti, S., 2010b. ^{68}Ga-DOTANOC PET/CT Clinical Impact in Patients with Neuroendocrine Tumors. J. Nucl. Med. 51, 669-673.

Ambrosini, V., Campana, D., Nanni, C., Cambioli, S., Tomassetti, P., Rubello, D., Fanti, S., 2012. Is ^{68}Ga-DOTA-NOC PET/CT indicated in patients with clinical, biochemical or radiological suspicion of neuroendocrine tumour? Eur. J. Nucl. Med. Mol. Imaging. 39, 1278-1283.

Antunes, P., Ginj, M., Zhang, H., Waser, B., Baum, R.P., Reubi, J.C., Maecke, H., 2007. Are radiogallium-labelled DOTA-conjugated somatostatin analogues superior to those labelled with other radiometals? Eur. J. Nucl. Med. Mol. Imaging. 34, 982-993.

Asti, M., De Pietri, G., Fraternali, A., Grassi, E., Sghedoni, R., Fioroni, F., Roesch, F., Versari, A., Salvo, D., 2008. Validation of $^{68}Ge/^{68}Ga$ generator processing by chemical purification for routine clinical application of ^{68}Ga-DOTATOC. Nucl. Med. Biol. 35, 721-724.

Asti, M., Iori, M., Capponi, P.C., Rubagotti, S., Fraternali, A., Versari, A., 2015. Development of a simple kit-based method for preparation of pharmaceutical-grade ^{68}Ga-DOTATOC. Nucl Med Commun. 36(5), 502-510.

Bozkurt, M.F., Virgolini, I., Balogova, S., Beheshti, M., Rubello, D., Decristoforo, C., Ambrosini, V., Kjaer, A., Delgado-Bolton, R., Kunikowska, J., Oyen, W.J.G., Chiti, A., Giammarile, F., Fanti, S., 2017. Guideline for PET/CT imaging of neuroendocrine neoplasms with ^{68}Ga-DOTA-conjugated somatostatin receptor targeting peptides and ^{18}F-DOPA. Eur. J. Nucl. Med. Mol. Imaging. 44(9), 1588-1601.

Breeman, W.A.P., De Jong, M., De Blois, E., Bernard, B.F., Konijnenberg, M., Krenning, E.P., 2005. Radiolabelling DOTA-peptides with ^{68}Ga. Eur. J. Nucl. Med. Mol. Imaging. 32, 478-485.

Breeman, W.A.P., Verbruggen, A.M., 2007. The ^{68}Ge/^{68}Ga generator has high potential, but when can we use ^{68}Ga-labelled tracers in clinical routine? Eur. J. Nucl. Med. Mol. Imaging. 34(7), 978-981.

Breeman, W.A.P., de Blois, E., Chan, H.S., Konijnenberg, M., Kwekkeboom, D.J., Krenning, E.P., 2011. ^{68}Ga-labeled DOTA-Peptides and ^{68}Ga-labeled radiopharmaceuticals for positron emission tomography: Current status of research, clinical applications, and future perspectives. Semin Nucl Med. 41, 314-321.

Chakravarty, R., Shukla, R., Ram, R., Venkatesh, M., Dash, A., Tyagi, A.K., 2010. Nanoceria-PAN composite-based advanced sorbent material: A major step forward in the field of clinical-grade ^{68}Ge/^{68}Ga generator. ACS Appl Mater Interfaces. 2, 2069-2075.

Conry, B.G., Papathanasiou, N.D., Prakash, V., Kayani, I., Caplin, M., Mahmood, S., Bomanji, J.B., 2010. Comparison of ^{68}Ga-DOTATATE and ^{18}F-fluorodeoxyglucose PET/CT in the detection of recurrent medullary thryroid carcinoma. Eur. J. Nucl. Med. Mol. Imaging. 37, 49-57.

Das, S.S., Chattopadhyay, S., Alam, M.N., Barua, L., Das, M.K., 2013. Preparation and evaluation of SnO_2-based ^{68}Ge/^{68}Ga generator made from ^{68}Ge produced through ^{nat}Zn($\propto$,χn) reaction. Appl Radiat Isot. 79, 42-47.

Das, T., Bhadwal, M., Sarma, H.D., Banerjee, S., 2014. Formulation and radiochemical evaluation of a freeze-dried mixed peptide kit for the preparation of ^{68}Ga-labeled peptides for PET imaging of somatostatin receptor positive neuroendocrine cancers. J Radioanal Nucl Chem. 302, 1259-1264.

Davids, C., 2017. Monitoring various eluate characteristics of the iThemba LABS SnO_2-based $^{68}Ge/^{68}Ga$ generator over time and validation of quality control methods for the radiochemical purity assessment of ^{68}Ga-labelled DOTA peptide formulations. Accessible at http://scholar.sun.ac.za/.

De Blois, E., Chan, H.S., Naidoo, C., Prince, D., Krenning, E.P., Breeman, W.A.P., 2011. Characteristics of SnO_2-based $^{68}Ge/^{68}Ga$ generator and aspects of radiolabelling DOTA-peptides. Appl Radiat Isot. 69, 308-315.

Decristoforo, C., Knopp, R., von Guggenberg, E., Rupprich, M., Dreger, T., Hess, A., Virgolini, I., Haubner, R., 2007. A fully automated synthesis for the preparation of ^{68}Ga-labelled peptides. Nucl Med Commun. 28, 870-875.

Decristoforo, C., Pickett, R.D., Verbruggen, A., 2012. Feasibility and availability of ^{68}Ga-labelled peptides. Eur. J. Nucl. Med. Mol. Imaging. 39(1), S31-S40.

EMA, 2017. SomaKit TOC: EPAR - Product Information. [online]. Retrieved 1 December 2017: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/004140/WC500221851.pdf

European Pharmacopoeia 8.0. Gallium (^{68}Ga) chloride solution for radiolabelling, 2013. Eur Pharm. 1060-1062.

European Pharmacopoeia 8.0. Gallium (^{68}Ga) Edotreotide injection, 2013. Eur Pharm. 1062-1064.

Fani, M., André, J.P., Maecke, H.R., 2008. ^{68}Ga-PET: a powerful generator-based alternative to cyclotron-based PET radiopharmaceuticals. Contrast Media Mol Imaging. 3, 67-77.

FDA, 2016. Prescribing Information NETSPOT [online]. Retrieved 1 December 2017: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/208547s000lbl.pdf.

Green, M.A., Welch, M.J., 1989. Gallium radiopharmceutical chemistry. Nucl. Med. Biol. 16(5), 435-448.

Henze, M., Schuhmacher, J., Hipp, P., Kowalski, J., Becker, D.W., Doll, J., Mäcke, H.R., Hofmann, M., Debus, J., Haberkorn, U., 2001. PET imaging of somatostatin receptors using [^{68}Ga]DOTA-D-Phe^{1}-Tyr^{3}-Octreotide: first results in patients with meningiomas. J. Nucl. Med. 42, 1053-1056.

Hofmann, M., Maecke, H., Börner, A.R., Weckesser, E., Schöffski, P., Oei, M.L., Schumacher, J., Henze, M., Heppeler, A., Meyer, G.J., Knapp, W.H., 2001. Biokinetics and imaging with the somatostatin receptor PET radioligand ^{68}Ga-DOTATOC: preliminary data. Eur J Nucl Med. 28, 1751-1757.

Jindal, T., Kumar, A., Venkitaraman, B., Dutta, R., Kumar, R., 2010. Role of ^{68}Ga-DOTATOC PET/CT in the evaluation of primary pulmonary carcinoids. Korean J. Intern. Med. 25, 386-391.

Kayani, I., Bomanji, J.B., Groves, A., Conway, G., Gacinovic, S., Win, T., Dickson, J., Caplin, M., Ell, P.J., 2008. Functional imaging of neuroendocrine tumors with combined PET/CT using ^{68}Ga-DOTATATE (Dota-$_{D}$Phe^{1}, Tyr^{3}-octreotate) and ^{18}F-FDG. Cancer. 112(11), 2447-2455.

Kibbe, A.H., 2009. Acetone monograph. In: Rowe, R.C., Sheskey, P.J., Quinn, M.E., (Eds), Handbook of Pharmaceutical Excipients, 6th edition. London and Chicago: Pharmaceutical Press. 7-8.

Kowalski, J., Henze, M., Schuhmacher, J., Mäcke, H.R., Hoffmann, M., Haberkorn, U., 2003. Evaluation of positron emission tomography imaging using [^{68}Ga]-DOTA-D Phe^{1}-Tyr^{3}-Octreotide in comparison to [^{111}In]-DTPAOC SPECT: first results in patients with neuroendocrine tumors. Mol Imaging Biol. 5, 42-48.

Krenning, E.P., Kwekkeboom, D.J., Bakker, W.H., Breeman, W.A.P., Kooij, P.P.M., Oei, H.Y., van Hagen, M., Postema, P.T.E., de Jong, M., Reubi, J.C., et al., 1993. Somatostatin receptor scintigraphy with [^{111}In-DTPA-D-Phe^{1}]- and [^{123}I-Tyr^{3}]-octreotide: the Rotterdam experience with more than 1000 patients. Eur J Nucl Med. 20, 716-731.

Kroiss, A., Putzer, D., Uprimny, C., Decristoforo, C., Gabriel, M., Santner, W., Kranewitter, C., Warwitz, B., Waitz, D., Kendler, D., Virgolini, I.J., 2011. Functional imaging in phaeochromocytoma and neuroblastoma with ^{68}Ga-DOTA-Tyr^{3}-octreotide positron emission tomography and ^{123}I-metaiodobenzylguanidine. Eur. J. Nucl. Med. Mol. Imaging. 38, 865-873.

Kwekkeboom, D.J., Kam, B.L., van Essen, M., Teunissen, J.J.M., van Eijck, C.H.J., Valkema, R., de Jong, M., de Herder, W.W., Krenning, E.P., 2010. Somatostatin receptor-based imaging and therapy of gastroenteropancreatic neuroendocrine tumors. Endocr. Relat. Cancer. 17, R53-R73.

Loc'h, C., Mazièré, B., Comar, D., 1980. A new generator for ionic Gallium-68. J. Nucl. Med. 21, 171-173.

Loktionova, N.S., Belozub, A.N., Filosofov, D.V., Zhernosekov, K.P., Wagner, T., Türler, A., Rösch, F., 2011. Improved column-based radiochemical processing of the generator produced ^{68}Ga. Appl Radiat Isot. 69(7), 942-946.

Maecke, H., Hofmann, M., Haberkorn, U., 2005. ^{68}Ga-labeled peptides in tumor imaging. J. Nucl. Med. 46, 172S-178S.

Martin, R., Jüttler, S., Müller, M., Wester, H., 2014. Cationic eluate pretreatment for automated synthesis of [^{68}Ga]CPCR4.2. Nucl. Med. Biol. 41, 84-89.

Meyer, G.-J., Hofmann, M., Kühn, J., Mäcke, H., Knapp, W.H., 2003a. ^{68}Ga-labelled DOTA-derivatised peptide-ligands. J Labelled Comp Radiopharm. 46, S273.

Meyer, G.-J., Hofmann, M., Schumacher, J., Harms, T., Knapp, W.H., 2003b. Production of highly concentrated ^{68}Ga from ^{68}Ge/^{68}Ga generators for labelling of peptides at nanomolar levels. J Labelled Comp Radiopharm. 46, S272.

Meyer, G.-J., Mäcke, H., Schuhmacher, J., Knapp, W.H., Hofmann, M., 2004. ^{68}Ga-labelled DOTA-derivatised peptide ligands. Eur. J. Nucl. Med. Mol. Imaging. 31, 1097-1104.

Mojtahedi, A., Thamake, S., Tworowska, I., Ranganathan, D., Delpassand, E.S., 2014. The value of ^{68}Ga-DOTATATE PET/CT in diagnosis and management of neuroendocrine tumors compared to current FDA approved imaging modalities: a review of literature. Am J Nucl Med Mol Imaging. 4(5), 426-434.

Mu, L., Hesselmann, R., Oezdemir, U., Bertschi, L., Blanc, A., Dragic, M., Löffler, D., Smuda, C., Johayem, A., Schibli, R., 2013. Identification, characterization and suppression of side-products formed during the synthesis of high dose ^{68}Ga-DOTA-TATE. Appl Radiat Isot. 76, 63-69.

Müller, D., Klette, I., Baum, R.P., 2011. The combined cationic-anionic purification of the ^{68}Ge/^{68}Ga generator eluate for the labelling of fragile peptides. Abstracts of Poster Presentations (Chemistry). World J Nucl Med. 10 (P-009), 73-89.

Mueller, D., Klette, I., Baum, R.P., Gottschaldt, M., Schultz, M.K., Breeman, W.A.P., 2012. Simplified NaCl based ^{68}Ga concentration and labeling procedure for rapid synthesis of ^{68}Ga radiopharmaceuticals in high radiochemical purity. Bioconjug. Chem. 23(8), 1712-1717.

Mukherjee, A., Pandey, U., Chakravarty, R., Sarma, H.D., Dash, A., 2014a. Development of single vial kits for preparation of ^{68}Ga-labelled peptides for PET imaging of neuroendocrine tumours. Mol Imaging Biol. 16, 550-557.

Mukherjee, A., Korde, A., Sarma, H.D., Samuel, G., 2014b. Single vial formulation for theranostic radiopharmaceutical preparation. J Radioanal Nucl Chem. 302, 889-894.

Mukherjee, A., Pandey, U., Chakravarty, R., Sarma, H.D., Dash, A., 2014c. Single vial kit formulation for preparation of PET radiopharmaceutical: ^{68}Ga-DOTA-TOC. J Radioanal Nucl Chem. 302, 1253-1258.

Neirinckx, R.D., Davis, M.A., 1979. Potential column chromatography generators for ionic Ga-68. I. Inorganic substrates. J. Nucl. Med. 20 (10), 1075-1079.

Neirinckx, R.D., Davis, M.A., 1980. Potential column chromatography for ionic Ga-68. II: Organic ion exchangers as chromatographic supports. J. Nucl. Med. 21, 81-83.

Ocak, M., Antretter, M., Knopp, R., Kunkel, F., Petrik, M., Bergisadi, N., Decristoforo, C., 2010. Full automation of ^{68}Ga labelling of DOTA-peptides including cation exchange prepurification. Appl Radiat Isot. 68, 297-302.

Oehlke, E., Le, V.S., Lengkeek, N., Pellegrini, P., Jackson, T., Greguric, I., Weiner, R., 2013. Influence of metal ions on the ^{68}Ga-labeling of DOTATATE. Appl Radiat Isot. 82, 232-238.

Oehlke, E., Lengkeek, N.A., Le, V.S., Pellegrini, P.A., Greguric, I., Weiner, R., 2016. The role of additives in moderating the influence of Fe(III) and Cu(II) on the radiochemical yield of [^{68}Ga(DOTATATE)]. Appl Radiat Isot. 107, 13-16.

Patrascu, I., Niculae, D., Lungu, V., Ursu, I., Iliescu, M., Tuta, C., Antohe, A., 2011. The purification and the quality control of ^{68}Ga eluates from ^{68}Ge/^{68}Ga generator. Rom Rep Phys. 63(4), 988-996.

Prasad, V., Ambrosini, V., Hommann, M., Hoersch, D., Fanti, S., Baum, R.P., 2010. Detection of unknown primary neuroendocrine tumours (CUP-NET) using ^{68}Ga-DOTA-NOC receptor PET/CT. Eur. J. Nucl. Med. Mol. Imaging. 37, 67-77.

Reubi, J.C., 1997. Regulatory peptide receptors as molecular targets for cancer diagnosis and therapy. Q J Nucl Med. 41(2), 63-70.

Reubi, J.C., Schär, J.C., Waser, B., Wenger, S., Heppeler, A., Schmitt, J.S., Mäcke, H.R., 2000. Affinity profiles for human somatostatin receptor subtypes SST1-SST5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use. Eur J Nucl Med. 27, 273-282.

Reubi, J.C., 2003. Peptide receptors as molecular targets for cancer diagnosis and therapy. Endocr. Rev. 24(4), 389-427.

Roesch, F., Zhernosekov, K., Filosofov, D., Jahn, M., Jennewein, W., Baum, R., Bihl, H., 2006. Processing of Ge-68/Ga-68 generator eluates for labeling of biomolecules via bifunctional chelators. J. Nucl. Med. 47(1), 162P.

Roesch, F., Riss, P.J., 2010. The renaissance of the ^{68}Ge/^{68}Ga radionuclide generator initiates new developments in ^{68}Ga radiopharmaceutical chemistry. Curr Top Med Chem. 10, 1633-1668.

Roesch, F., 2012. Maturation of a key resource – The Germanium-68/Gallium-68 generator: Development and new insights. Curr Radiopharm. 5, 202-211.

Rossouw, D.D., Breeman, W.A.P., 2012. Scaled-up radiolabelling of DOTATATE with ^{68}Ga eluted from a SnO_2-based ^{68}Ge/^{68}Ga generator. Appl Radiat Isot. 70, 171-175.

Schultz, M.K., Mueller, D., Baum., R.P., Watkins, G.L., Breeman, W.A.P., 2013. A new automated NaCl based robust method for routine production of gallium-68 labeled peptides. Appl Radiat Isot. 76, 46-54.

Seemann, J., Eppard, E., Waldron, B.P., Ross, T.L., 2015. Cation exchange-based post-processing of ^{68}Ga eluate: A comparison of three solvent systems for labelling of DOTATOC, NO2AP^{BP} and DATA^{m}. Appl Radiat Isot. 98, 54-59.

Šimeček, J., Hermann, P., Wester, H.-J., Notni, J., 2013. How is ^{68}Ga labeling of macrocyclic chelators influenced by metal ion contaminants in ^{68}Ge/^{68}Ga Generator eluates? ChemMedChem. 8, 95-103.

Sudbrock, F., Fischer, T., Zimmermanns, B., Guliyev, M., Dietlein, M., Drzezga, A., Schomäcker, K., 2014. Characterization of SnO_2-based ^{68}Ge/^{68}Ga generators and ^{68}Ga-DOTATATE preparations: radionuclide purity, radiochemical yield and long-term constancy. Eur. J. Nucl. Med. Mol. Imaging Research. 4, 36.

Velikyan, I., Beyer, G.J., Långström, B., 2004. Microwave-supported preparation of ^{68}Ga bioconjugates with high specific radioactivity. Bioconjug. Chem. 15(3), 554-560.

Velikyan, I., Beyer, G.J., Bergström-Pettermann, E., Johansen, P., Bergström, M., Långström, B., 2008. The importance of high specific radioactivity in the performance of ^{68}Ga-labeled peptide. Nucl. Med. Biol. 35, 529-536.

Velikyan, I., Sundin, A., Eriksson, B., Lundqvist, H., Sörensen, J., Bergström, M., Långström, B., 2010. In vivo binding of [^{68}Ga]-DOTATOC to somatostatin receptors in neuroendocrine tumours — impact of peptide mass. Nucl. Med. Biol. 37, 265-275.

Velikyan, I., Xu, H., Nair, M., Hall, H., 2012. Robust labeling and comparative preclinical characterization of DOTA-TOC and DOTA-TATE. Nucl. Med. Biol. 39, 628-639.

Velikyan, I., 2015. ^{68}Ga-based radiopharmaceuticals: Production and application relationship. Molecules. 20, 12913-12943.

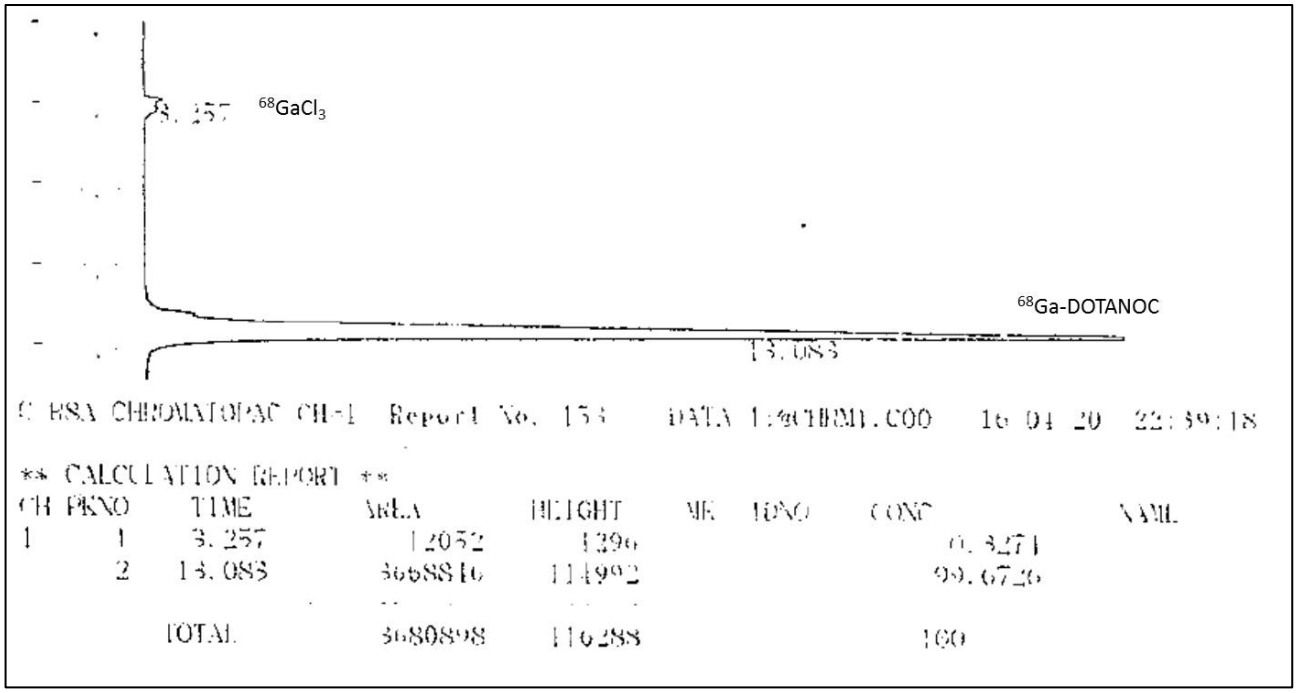
Wild, D., Schmitt, J.S., Ginj, M., Mäcke, H.R., Bernard, B.F., Krenning, E., De Jong, M., Wenger, S., Reubi, J.C., 2003. DOTA-NOC, a high-affinity ligand of somatostatin receptor subtypes 2, 3 and 5 for labelling with various radiometals. Eur. J. Nucl. Med. Mol. Imaging. 30(10), 1338-1347.

Wild, D., Bomanji, J.B., Benkert, P., Maecke, H., Ell, P.J., Reubi, J.C., Caplin, M.E., 2013. Comparison of ^{68}Ga-DOTANOC and ^{68}Ga-DOTATATE PET/CT within patients with gastroenteropancreatic neuroendocrine tumors. J. Nucl. Med. 54(3), 364-372.

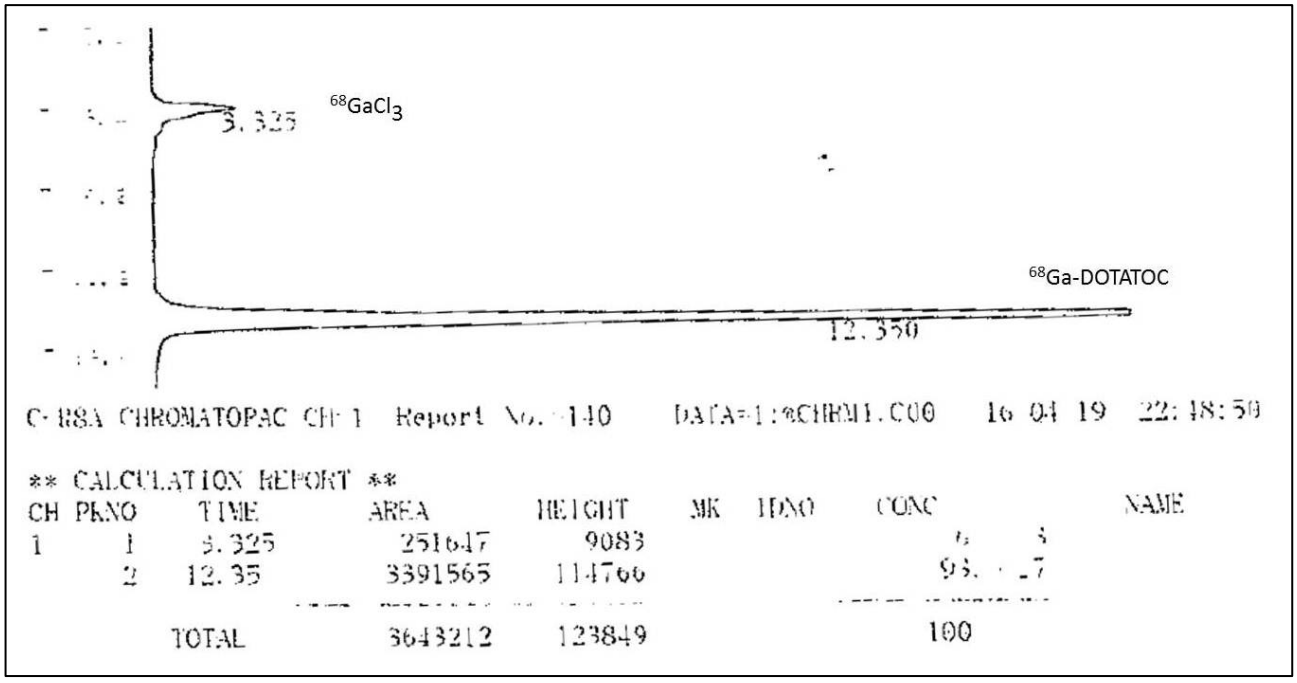
Zhernosekov, K.P., Filosofov, D.V., Baum, R.P., Aschoff, P., Bihl, H., Razbash, A.A., Jahn, M., Jennewein, M., Rösch, F., 2007. Processing of generator-produced ^{68}Ga for medical application. J. Nucl. Med. 48, 1741-1748.

Annex 1

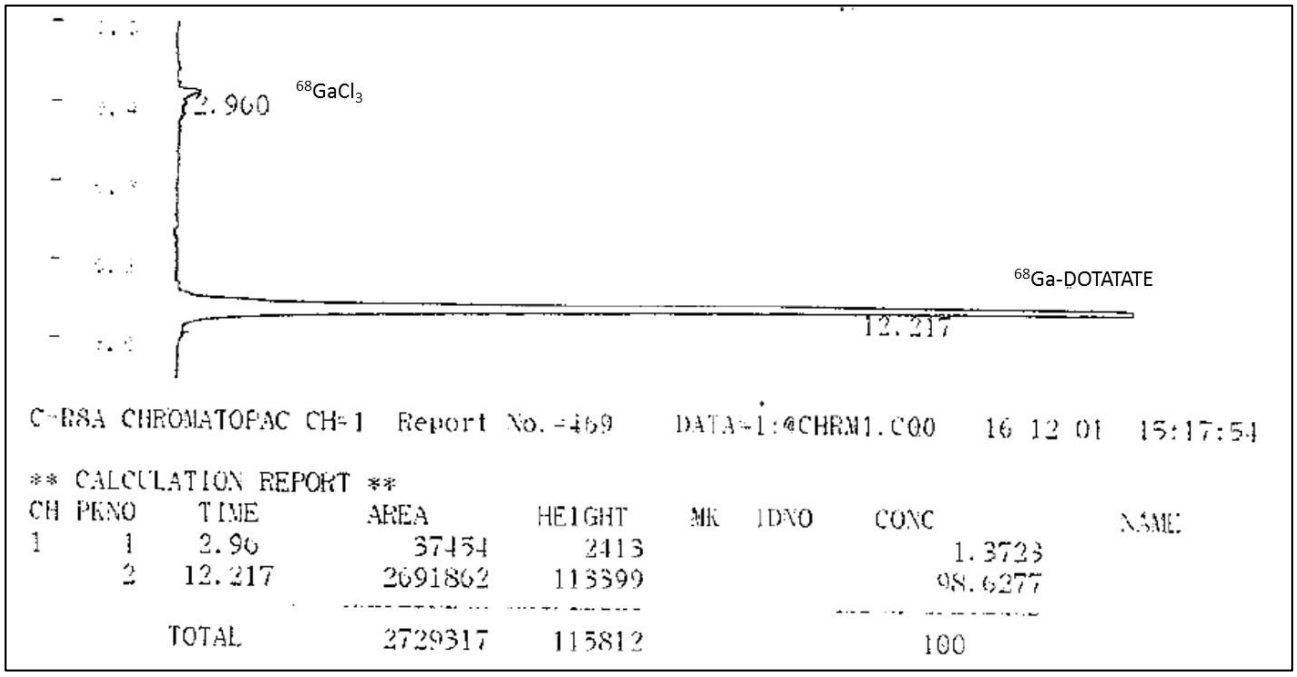
Examples of chromatograms



Chromatogram 1: HPLC chromatogram of ^{68}Ga-DOTANOC before Sep Pak purification



Chromatogram 2: HPLC chromatogram of ^{68}Ga-DOTATOC before Sep Pak purification

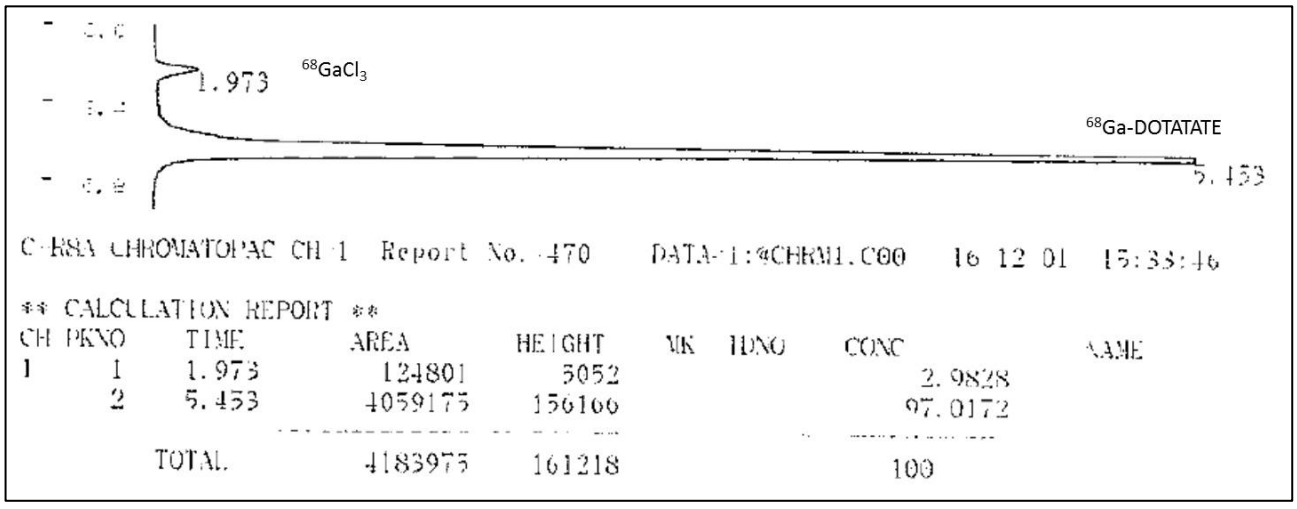


C-RSA CHROMATOPAC CH-1 Report No.-469 DATA-1:@CHRM1.C00 16 12 01 15:17:54

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	2.96	37454	2413			1.3723	
	2	12.217	2691862	113399			98.6277	
		TOTAL	2729317	115812			100	

Chromatogram 3: HPLC chromatogram of ^{68}Ga-DOTATATE before Sep Pak purification



C-RSA CHROMATOPAC CH-1 Report No.-470 DATA-1:@CHRM1.C00 16 12 01 15:33:46

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	1.973	124801	5052			2.9828	
	2	5.453	4059175	156166			97.0172	
		TOTAL	4183975	161218			100	

Chromatogram 4: iTLC chromatogram of ^{68}Ga-DOTATATE before Sep Pak purification

Annex 2

Publications and presentations arising from the work presented in this Dissertation

Published papers

- Prince, D., Rossouw, D., Davids, C., Rubow, S., 2017. Development and evaluation of user-friendly single vial DOTA-peptide kit formulations, specifically designed for radiolabelling with ^{68}Ga from a tin dioxide ^{68}Ge/^{68}Ga generator. Mol Imaging Biol. 19, 817-824

List of planned publications

At least two further publications have been identified from this study:

- Optimization of a labelling and kit preparation method for ^{68}Ga-labelled DOTATATE, using cation exchange resin purified ^{68}Ga eluates obtained from a tin dioxide ^{68}Ge/^{68}Ga generator
- An adapted cationic resin pre-treatment method for the concentration and purification of ^{68}Ga eluates from a tin dioxide ^{68}Ge/^{68}Ga generator

Presentations

The following poster presentation was made at the South African Society of Nuclear Medicine (SASNM) 17th Biennial Congress, September 2016:

Influence of non-elution of the ^{68}Ge/^{68}Ga generator on the characteristics and radiolabelling quality of ^{68}Ga eluates