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

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Abstract

PET imaging with gallium-68 (^{68}Ga) has become widely used due to the availability of ^{68}Ge/^{68}Ga generators and DOTA-derivatised peptide ligands for radiolabelling. The purpose of this study was to monitor the eluate of two iThemba LABS ^{68}Ge/^{68}Ga generators over a period of 12 months to ascertain whether all quality parameters of the ^{68}Ga eluate remained stable and to validate different analytical methods used to determine the radiochemical purity of ^{68}Ga-labelled peptides.

Two 1850 MBq (50 mCi) generators were eluted daily with 0.6 M HCl and metal contaminants, ^{68}Ge breakthrough, ^{68}Ga yield, pH, sterility and endotoxin concentrations were determined on a monthly basis. The radiochemical purity of ^{68}Ga-labelled peptides was ascertained using high performance liquid chromatography (HPLC) and instant thin layer chromatography (iTLC). iTLC experiments were performed using both dried and undried iTLC plates. iTLC was also carried out on labelled peptide solution that was spiked with $^{68}GaCl_3$. These results were also compared with those using HPLC.

After 12 months the ^{68}Ga yields, total metal contaminants, sterility and endotoxin concentration remained within European Pharmacopoeial limits. The ^{68}Ge breakthrough increased as the generator aged. This can however be minimised by fractionated elution and post-labelling processing of the eluate by anion or cation exchange chromatography.

Separation between $^{68}GaCl_3$ and ^{68}Ga-labelled peptides was obtained using both 0.1 M citrate buffer pH 5.0 (mobile phase 1) and 1 M ammonium acetate : methanol (1:1) (mobile phase 2). The results also showed that the distribution of radioactivity on the iTLC strip could be determined using a dose calibrator when a TLC scanner is not available. Experiments performed using both undried and dried iTLC-SG chromatography paper, demonstrated that despite the statistically significant difference between the sets of results, in practice either undried or dried iTLC may be used. When purified ^{68}Ga-labelled peptides were spiked with 2% of $^{68}GaCl_3$, separation between the two was obtained on both HPLC and iTLC. However, iTLC underestimated and HPLC overestimated $^{68}GaCl_3$ content. Of the two iTLC methods investigated, the method using mobile phase 2 was able to separate colloidal ^{68}Ga impurities from the ^{68}Ga-labelled peptides while the method using mobile phase 1 and the HPLC method could not.

In conclusion, the iThemba LABS $^{68}Ge/^{68}Ga$ generator can be considered stable and of use for up to one year after its manufacture. Both the iTLC method and the HPLC method could detect $^{68}GaCl_3$ amounts less than 2%. The pharmacopoeia states that ^{68}Ga must be less than 3 % on iTLC and less than 2 % on HPLC. Either dried or undried iTLC strips can be used and if a radio-TLC scanner is not available, the iTLC strips developed with mobile phase 1 can be cut at a suitable distance from the origin and the activity on each section can be read in a dose calibrator. iTLC chromatography using ammonium acetate/methanol seems to be the optimal system for routine analysis of ^{68}Ga labelled DOTA-peptides, as it separates both $^{68}GaCl_3$ and colloidal impurities from the labelled peptides and is a fast and easy technique.

Opsomming

PET beelding met gallium-68 (^{68}Ga) word deesdae algemeen aangewend as gevolg van die beskikbaarheid van ^{68}Ge/^{68}Ga generators en DOTA-afgeleide peptied ligande vir radiomerking. Die doelstelling van hierdie studie was om die eluate van twee iThemba LABS ^{68}Ge/^{68}Ga generators oor ‘n 12 maande periode te moniteer om vas te stel of al die gehalteparameters van die ^{68}Ga eluaat stabiel bly. ‘n Verdere doelstelling was om verskillende analitiese metodes vir bepaling van die radiochemiese suiwerheid van ^{68}Ga-gemerkte peptiede te bekragtig.

Twee 1850 MBq (50 mCi) generators is daagliks met 0.6M HCl ge-elueer en bepalings van metaalonsuiwerhede, ^{68}Ge deurbraak, ^{68}Ga opbrengste, pH, steriliteit en endotoksien konsentrasie is maandeliks herhaal. Die radiochemiese suiwerhede van ^{68}Ga-gemerkte peptiede is met behulp van hoèdoeltreffendheid-vloeistofchromatografie (HDVC) en kits dunlaag chromatografie of sg. *instant thin layer chromatography* (iTLC) bepaal. iTLC eksperimente is uitgevoer met beide gedroogde en ongedroogde iTLC papier. iTLC is ook uitgevoer op gemerkte peptied monsters wat doelbewus met klein hoeveelhede $^{68}GaCl_3$ gekontamineer is. Hierdie resultate is vergelyk met HDVC resultate van dieselfde monsters.

Die ^{68}Ga opbrengste, totale metaalonsuiwerhede, steriliteit en endotoksien konsentrasie het na ‘n periode van 12 maande binne die grense van die Europese Farmakopee gebly. Die ^{68}Ge deurbraak het toegeneem met veroudering van die generator maar dit kan beperk word deur gefraksioneerde eluering of prosessering van die eluaat met behulp van anioon- of katioon-uitruilchromatografie.

Skeiding tussen $^{68}GaCl_3$ en ^{68}Ga-gemerkte peptiede is verkry met die gebruik van beide 0.1 M sitraat buffer pH 5 (mobiele fase 1) en 1 M ammonium asetaat : metanol (1:1) (mobiele fase 2). Die resultate het getoon dat die verspreiding van radioaktiwiteit op ‘n iTLC strook met behulp van ‘n dosiskalibreerder bepaal kan word wanneer ‘n TLC skandeerder nie beskikbaar is nie. Die eksperimente met vooraf gedroogde sowel as ongedroogde iTLC strokies het getoon dat, ten spyte van statisties betekenisvolle verskille tussen die resultate, beide in praktyk gebruik kan word. In gevalle waar gesuiwerde ^{68}Ga-gemerkte peptiede doelbewus gekontamineer is met 2% $^{68}GaCl_3$, is skeiding tussen die twee spesies verkry in beide HDVC en iTLC analises. $^{68}GaCl_3$ inhoud is egter onderskat met iTLC en oorskat met HDVC. Die metode waarin mobiele fase 2 gebruik is, was in staat om kolloïdale ^{68}Ga onsuiwerhede te skei van die ^{68}Ga-gemerkte peptiede, terwyl mobiele fase 1 en die HDVC metodes dit nie kon doen nie.

Ter samevatting, die iThemba LABS $^{68}Ge/^{68}Ga$ generator kan as stabiel beskou word en vir ‘n periode van tot een jaar na vervaardiging gebruik word. Beide die HDVC en iTLC metodes kon ^{68}Ga hoeveelhede van minder as 2% bepaal. Die Europese Farmakopee skryf voor dat ^{68}Ga laer as 3 % moet wees met iTLC en laer as 2 % met HDVC. Gedroogde of ongedroogde iTLC papier kan gebruik word en indien ‘n iTLC radioskandeerder nie beskikbaar is nie, kan iTLC stroke wat met mobiele fase 1 ontwikkel is, ‘n geskikte afstand vanaf die oorsrong deurgesny word en die aktiwiteit op elke deel in ‘n dosiskalibreerder gelees word. iTLC met ammonium asetaat/metanol as mobiele fase, blyk die optimale sisteem vir roetine-analise van ^{68}Ga-gemerkte peptiede te wees, omdat dit beide $^{68}GaCl_3$ en kolloïdale ^{68}Ga

onsuiwerhede van die gemerkte peptiede kan skei en ook ‘n vinnige en maklike tegniek is.

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Chapter 1: Introduction

Over the past twenty years, Positron Emission Tomography (PET) has become an accepted tool for diagnosing disease. [1] The limitations of fluorine-18 fluorodeoxyglucose (^{18}F-FDG), which has been widely used for PET applications, prompted the development of new PET radiopharmaceuticals such as those labelled with gallium-68 (^{68}Ga) [2]. ^{68}Ga-labelled radiopharmaceuticals have proved to be significant and relevant as PET tracers for the detection and management of certain tumours, including neuroendocrine tumours [2]. ^{68}Ga is obtained from ^{68}Ge/^{68}Ga generators, which have been available since the beginning of the twenty first century. The recent development of a number of peptide ligands has resulted in the increased use of the ^{68}Ge/^{68}Ga generator [2].

There are five different generators on the market [3, 4]. The columns of these generators are either titanium dioxide (TiO_2), tin dioxide (SnO_2) or organic resin based. For clinical applications, the eluates of these generators have to be of medicinal quality i.e. low germanium-68 (^{68}Ge) breakthrough, low metal ion content and sterile and apyrogenic. A narrow elution profile is essential as it gives the most activity in the smallest volume of eluate. These properties must remain stable for the shelf life of the generator [3]. In addition, ^{68}Ga-labelled peptides should also comply with the necessary quality requirements such as radiochemical purity, in order to be suitable for use as radiopharmaceuticals [2]. Radiochemically impure labelled peptides, such as those that are contaminated with free gallium-68 (^{68}Ga), could result in low quality PET images and increase radiation exposure of patients.

The aims of this research study are to evaluate the ^{68}Ga eluate obtained from the iThemba LABS tin dioxide-based ^{68}Ge/^{68}Ga generator over a period of twelve months in order to ascertain whether the quality of the eluate remains stable, and to validate the quality control methods currently published in literature and used in radiopharmaceutical laboratories all over the world to determine the radiochemical purity of ^{68}Ga-labelled DOTA peptides.

Chapter 2: Literature Review and Problem Statement

LITERATURE REVIEW

2.1 PET Radiopharmaceuticals

Since the introduction of PET, ^{18}F-FDG has been a work-horse of major PET facilities across the globe. FDG is a glucose analogue that is taken up by cells that have a high metabolic turnover and is therefore useful for detecting malignant tumours. It is commonly used in staging, re-staging, assessing response to therapy and in regular follow up of oncology patients. However, ^{18}F-FDG does have limitations including [1]:

- Some highly differentiated cancers, including prostate carcinoma, have a low growth rate and may therefore not be suitable for ^{18}F-FDG scans;
- With ^{18}F-FDG it may be difficult to evaluate lesions in or close to tissues that normally show high FDG concentrations due to metabolism of the radiopharmaceutical e.g. brain or bladder; and
- ^{18}F-FDG is not specific for malignancies, as it also accumulates in infective or inflammatory lesions.

These shortcomings triggered the development of many new positron emitting radiopharmaceuticals. ^{68}Ga-labelled peptides such as somatostatin analogues, minigastrin, bombesin and RGD-based peptides, offer a solution to these shortcomings with the combined advantage of the short half-life (that is nevertheless long enough to allow chemical manipulations) and availability of ^{68}Ga from a ^{68}Ge/^{68}Ga generator [2].

2.2 ^{68}Ge/^{68}Ga Generator

A radionuclide generator consists of a system containing a parent radionuclide which decays to a daughter isotope with a shorter half-life. The daughter isotope can be eluted while the parent is retained. In the case of the ^{68}Ge/^{68}Ga generator, the parent radionuclide ^{68}Ge is in equilibrium with the daughter radionuclide ^{68}Ga. The generator provides a constant source of ^{68}Ga which, because of its short half-life, cannot be shipped over long distances. [3]. An ideal generator system has the following properties:

1. The eluate must be sterile and pyrogen free for clinical applications.
2. The daughter radionuclide (in this case ^{68}Ga) must differ chemically from the parent radionuclide (^{68}Ge) to enable separation by means of chromatography.
3. No aggressive chemical reactions should be required to retrieve the daughter radionuclide from the generator system.
4. The elution process should be very simple, with limited manipulation of radioactivity in order to protect the operator from excessive radiation exposure.
5. For diagnostic imaging the daughter radionuclide should be a gamma photon or positron emitter with a short half-life.
6. The parent radionuclide should have a sufficiently short physical half-life to allow fast re-growth of the daughter radionuclide after elution. At the same time, the half-life of the parent radionuclide must be sufficiently long to allow time for transport and to provide a long generator usage time.

7. The properties of the daughter radionuclide should be such that it can be used to prepare a range of radiolabelled compounds.
8. The daughter radionuclide should decay to a stable “granddaughter” to minimize exposure of patients, staff or the public.
9. To reduce radiation exposure of users of the generator, it should be contained in suitable shielding [3].

2.2.1 History of the ^{68}Ge/^{68}Ga Generator

The ^{68}Ge/^{68}Ga generator was first described by Gleason in 1960 [5]. He made reference to a “positron cow” where the daughter radionuclide with a shorter half-life would be available when needed while the parent radionuclide with the longer half-life was retained for “growth” of the daughter radionuclide. Gleason employed a solvent-extraction method with acetylacetone in cyclohexane for the separation of ^{68}Ga from ^{68}Ge.

In 1961 Green and Tucker [6] introduced a solid phase ion exchange extraction method for the separation of ^{68}Ga from ^{68}Ge. The generator comprised an Al_2O_3 support and was eluted with ethylenediaminetetraacetic acid (EDTA).

In 1964, Yano and Anger [7] described a generator with an alumina column that was eluted with 0.005 M EDTA. The ^{68}Ga-EDTA eluate could be used directly for clinical patient imaging, e.g. for localisation of brain tumours. The development of new radiopharmaceuticals, however, was prevented due to the complicated chemistry that was required to break down the stable ^{68}Ga-EDTA complex.

In 1979 Loch et al [8] developed a $^{68}Ge/^{68}Ga$ generator as a source of ionic ^{68}Ga. The generator consisted of a tin dioxide column and it was eluted with hydrochloric acid (HCl). The tin dioxide generator provided a sterile solution of ^{68}Ga in ionic form, ready for use in the preparation of many radiopharmaceuticals. Other ionic generators have $Al(OH)_3$, $Fe(OH)_3$, iron oxide, ZrO_2, TiO_2 and CeO_2 supports [3].

In 1981 Schumacher and Maier-Borst [9] prepared a generator using an ion exchange resin composed of pyrogallol and formaldehyde. The ^{68}Ga was easily eluted with HCl. Nakayama et al reported in 2003 that a styrene-divinyl-benzene copolymer, containing N-methylglucamine, was a suitable support for ^{68}Ge from which ^{68}Ga can be eluted with citric acid or phosphoric acid [10].

2.2.2 Current $^{68}Ge/^{68}Ga$ Generators

The generators that are currently on the market are either titanium dioxide (TiO_2), tin dioxide (SnO_2) or organic resin based. The TiO_2 based generators are manufactured by Cyclotron Ltd, Obninsk, Russia and Eckert and Ziegler, Germany. iThemba LABS in South Africa produces a SnO_2 based generator system. To prepare this generator, a gallium suboxide (GaO_2) target is bombarded with protons to produce the parent radionuclide ^{68}Ge via the nuclear reaction $^{69}Ga(p,2n)^{68}Ge$. The ^{68}Ge is then radiochemically separated from the Ga target by solvent extraction and loaded onto a column composed of a calcified SnO_2 resin [11]. A SiO_2/organic (organic matrix on a silica resin) generator which is eluted with 0.05 N HCl, is available from Isotope Technologies Garching (ITG) [3]. A fifth generator, Galli Eo is produced by IRE ELiT (the column material is not specified) [4]. The column matrices and eluents of the different $^{68}Ge/^{68}Ga$ generators are

summarised in Table 1. An overview of the properties of the generators that are commercially available is presented in Table 2. The TiO_2 generator from Cyclotron Ltd, Obninsk, Russia has an initial ^{68}Ga yield of approximately 80 % with a ^{68}Ge breakthrough of approximately $1\text{-}10^{-3}$ %. The ^{68}Ga yield decreases to about 50 % and the breakthrough to about 10^{-2} % after 200 elutions. The elution characteristics of the Eckert and Ziegler IGG100 generator are said to surpass that of the Cyclotron Ltd generator. The iThemba LABS SnO_2 generator has an optimum ^{68}Ga elution efficiency with 0.6 M HCl and the elution efficiency of the generator was found to decrease to about 50 % after 100 elutions [12].

In 2013, Rösch [13] projected that further developments in labelling techniques, clinical applications, improvements in resin materials to reduce ^{68}Ge breakthrough, new ligands and GMP-certified generators will result in increased use of the $^{68}Ge/^{68}Ga$ generators.

TABLE 1: Column matrices with corresponding eluents

(reproduced from Velikyan: ^{68}Ga-based Radiopharmaceuticals: Production and Application Relationship [3])

^{68}Ge/^{68}Ga Generator Column Matrix and Eluents	
Inorganic	
Matrix	Eluents
SnO_2	1 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	
Matrix	Eluents
Pyrogallol-formaldehyde	0.3 M HCl
Nanoceria-polyacrylonitrile	0.1 M HCl
N-methylglucamine	0.1 M HCl; 0.1 M NaOH; citrate; EDTA

TABLE 2: Commercially available generators and their properties

(reproduced from Velikyan: ^{68}Ga-based Radiopharmaceuticals: Production and Application Relationship [3] and producer specifications)

	Obninsk Cyclotron Co. Ltd. (marketed by Eckert and Ziegler)	Eckert & Ziegler IGG100 and IGG101 GMP; Pharm. Grade	I.D.B. Holland B.V. (iThemba LABS)	Isotope Technologies Garching (ITG)	IRE Elit
Column matrix	TiO_2	TiO_2	SnO_2	SiO_2/organic	Not specified
Eluent	0.1 M HCl	0.1 M HCl	0.6 M HCl	0.05 M HCl	0.1 M HCl
^{68}Ga Yield	not < 75 % for each elution	Greater than 60 % of nominal activity	Not less than 80 % at calibration time	Greater than 80 % on calibration date	70 – 75 % and ≥ 60 % after 12 months
^{68}Ge breakthrough	<0.005 % after 400 elutions	<0.001 %	< 0.001 % at calibration time	<0.005 %	≤ 0.001 %
Eluate volume	5 ml	5 ml	6 ml	4 ml	1.1 ml
Chemical impurity	Ga: <1 µg/37 MBq Ni < 1µg/37 MBq	Fe: <10 µg/GBq Zn: <10 µg/GBq	<10 ppm (Zn, Sn, Fe, Al,)	Only Zn from decay	Fe, Cu, Ga, Ni, Pb, Zn ≤ 10 µg/GBq individually
Weight	11.7 kg	10 kg and 14 kg	26 kg	16 kg	Not specified

2.2.3 Studies performed to evaluate the eluate of ^{68}Ge/^{68}Ga generators

The following studies were performed to evaluate the generator:

- In 1984, McElvany et al. [14] compared the elution profiles, yields, germanium breakthrough and metal contaminants of three different generator types over a period of one year. The generators were an

alumina/0.005 M EDTA $^{68}Ge/^{68}Ga$ generator, an alumina 0.1 M NaOH $^{68}Ge/^{68}Ga$ generator and a tin dioxide/1 M HCl $^{68}Ge/^{68}Ga$ generator. Elution yields were measured daily for one year. Eluates were measured immediately after elution and again after 24 to 48 hours using a NaI(Tl) scintillation detector to determine ^{68}Ge breakthrough. Metal contaminants were determined by means of emission spectroscopy.

- In 2008, Asti et al. [15] evaluated the eluates from a titanium dioxide generator over a period of 7 months. The generator was eluted 3 times a week and approximately 100 elutions were performed. The eluates were used to determine ^{68}Ga yield, ^{68}Ge breakthrough and metal contaminants.
- In 2013, Das et al. [16] published an evaluation of a tin dioxide generator over a period of six months. This generator was produced by the Board of Radiation and Isotope Technology, India. Random eluates were used to monitor elution efficiency, ^{68}Ge breakthrough and ^{65}Zn content.
- A study was published in 2014 by Sudbrock et al. [17] using four iThemba LABS SnO_2-based $^{68}Ge/^{68}Ga$ generators to evaluate the ^{68}Ge breakthrough over time. Three of the generators were evaluated over a period of nine months while the fourth generator was evaluated over a period of eight months. Three samples of eluate were measured repeatedly over a period of 7 months to obtain the decay curves. ^{68}Ge breakthrough was determined on 123 eluates and 115 ^{68}Ga-DOTATATE samples.
- Ebenhan et al. performed a retrospective analysis to evaluate the effect of eluate characteristics on several ^{68}Ga-labelled peptides over a prolonged period of generator use, but no data was provided [18].

- In 2016, Amor-Coarasa et al. [19] evaluated the performance of the ITG generator over a period of 1 year. The ^{68}Ge breakthrough was <0.006 % at the start and decreased to 0.001 %. A decrease in ^{68}Ge breakthrough with time is unique to this generator.
- A study to ascertain whether microorganisms survived in ^{68}Ga eluates and after the re-generation of cold columns which had been loaded with different microorganisms was performed by Petrik et al. [20]. A titanium dioxide generator was used for these experiments and the microorganisms used included *Staphylococcus aureus*, *Clostridium sporogenes*, *Helicobactor pylori*, *Deinococcus radiodurans*, *Aspergillus niger* and *Candida albicans*.

2.2.4 European Pharmacopoeia (Ph Eur) Specifications for ^{68}Ga Eluate

If the ^{68}Ga eluate is to be used for clinical applications it must meet the Ph Eur specifications below [21]:

Appearance:	Clear and colourless
Radionuclidic identity (half-life determination):	62 to 74 minutes
Radionuclidic identity (gamma-ray spectrometry):	511 and 1077 keV
Radionuclidic purity (gamma-ray spectrometry):	> 99.9 %
^{68}Ge breakthrough:	< 0.001 %
Radiochemical purity (TLC):	> 95 %
Microbiological quality:	sterile
Bacterial endotoxins:	< 175/V EU/ml*

* V = volume to be injected and EU = Endotoxin Units

pH:	< 2
Iron:	< 10 µg/GBq
Zinc:	< 10 µg/GBq

All the parameters, except the radionuclidic identity, which is confirmed only at the start of use of the generator, must be evaluated for the period of use. The pH of the eluate is not expected to change unless a different eluent is used.

2.3 Peptide Ligands

^{68}Ga allows excellent visualization of tumours and small metastases when labelled with a suitable ligand. ^{68}Ga can be coupled with peptides including somatostatin analogues and prostate specific membrane antigen (PSMA) antibody fragments. The ^{68}Ga-labelled somatostatin analogues DOTANOC, DOTATATE and DOTATOC (Figure 1) have been used clinically since the 1990's [22]. The ^{68}Ga-DOTA-conjugated peptides have a high affinity for somatostatin receptors which are over-expressed in neuroendocrine tumours (NETs) and therefore have great potential for the imaging of somatostatin receptor-expressing tumours by means of PET scans [23].

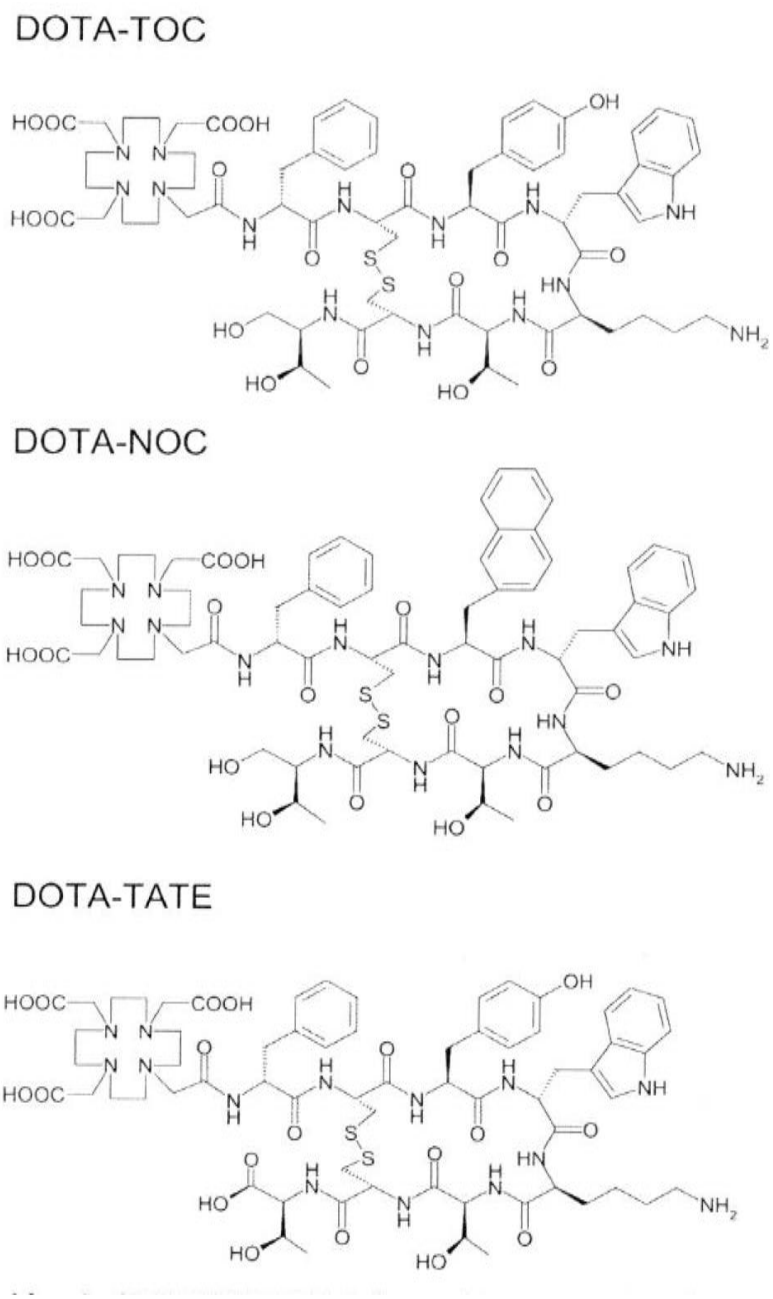

Figure 1: Structural Formula of DOTATATE, DOTATOC and DOTANOC (reproduced from Breeman et al. [24])

Somatostatin is a small peptide that binds to somatostatin receptors. It is found in neurones and endocrine cells, brain, pancreas and gastro-intestinal tract. The somatostatin receptors are expressed by many neuroendocrine and non-neuroendocrine cells of the body. Five different types of human somatostatin receptors have been identified and all the receptors are over expressed in

neuroendocrine tumours. Because somatostatin has a short biological half-life, more stable synthetic analogues have been developed. When radiolabelled with ^{68}Ga, somatostatin analogues enable *in vivo* visualisation of these tumours by means of somatostatin receptor scintigraphy using PET or SPET scans [23]. The molecular structure of the DOTA analogues of somatostatin, i.e. DOTATATE, DOTATOC and DOTANOC, enables rapid and efficient binding with ^{68}Ga at high specific activities [25].

Initial patient studies have demonstrated the potential of PET technology using ^{68}Ga-DOTANOC, ^{68}Ga-DOTATATE and ^{68}Ga-DOTATOC. They are used for diagnosis, staging, prognosis, therapy selection and response monitoring of NETs and other types of cancers and diseases. The major difference among these compounds is the slight difference in affinities to somatostatin subtypes. All tracers can bind to sst2 receptors, which is the predominant receptor type in NETs. DOTATOC and DOTANOC also bind to sst5 receptors, while only the DOTANOC analogue shows good affinity for sst3 receptors [26].

Tumour types that may be visualised with PET/CT, using ^{68}Ga-DOTA-conjugated somatostatin analogues, include gastro-entero-pancreatic tumours and sympatho-adrenal system tumours as well as several other carcinomas [27].

2.3.1 Chemistry of ^{68}Ga and peptide labelling

Gallium can exist in oxidation state Ga^{3+} and Ga^{1+}. ^{68}Ga is eluted with HCl in the form of Ga^{3+}, which is the only stable chemical form in aqueous solution. The free Ga^{3+} in acid solution binds with the peptide molecules during the radiolabelling reaction. At pH values up to 3, Ga^{3+} and $[Ga(H_2O)_6]^{3+}$ which is soluble, are formed. At pH values between 3 and 7, Ga^{3+} hydrolyses and forms a colloidal precipitate i.e. $Ga(OH)_3$. Gallate ions ($[Ga(OH)_4]^-$) are formed at pH values greater than 7.4. Colloids and gallate ions will not form complexes with peptide molecules and will result in low labelling yields. Labelling with ^{68}Ga is therefore pH sensitive and the pH of the reaction mixture has to be low to minimise the formation of colloids and gallate ions [12]. In a labelling mixture that is buffered with citrate, acetate or HEPES, the Ga is in the form of $[Ga(H_2O)_6]^{3+}$. In this form the Ga is inhibited from being hydrolysed to $Ga(OH)_3$. After radiolabelling it would be possible to find either unbound gallium chloride, or hydrolysed insoluble gallium hydroxide in the peptide preparation. Gallium chloride is almost entirely bound to transferrin after intravenous injection of very small amounts [28], from where it probably slowly distributes mostly to bones, lungs, kidney and spleen [29]. Like other colloids, insoluble gallium species are expected to accumulate in the liver, spleen and bone marrow [30]. Because Ga can exist in different forms, the methods used for analysis must be capable of detecting them. According to the European Pharmacopeia (Ph Eur) monograph for ^{68}Ga-DOTATATE [21], free ^{68}Ga must be less than 3 % on iTLC and less than 2 % on HPLC and not more than 3 % of the total radioactivity should be due to ^{68}Ga in colloidal form for TLC analysis.

2.3.2 Radiochemical Purity of DOTA-Peptides

Simple and reliable quality control (QC) analytical methods should be available for determining the integrity of the labelled peptides before administration to patients. The methods employed are usually high performance liquid chromatography (HPLC), which requires more complex equipment and relatively large volumes of mobile phases, and instant thin Layer chromatography (iTLC) because of its convenience and the short time within which it can be performed. The methods used to determine the radiochemical purity of ^{68}Ga-labelled peptides are summarized below.

2.3.2.1 TLC Methods

Di Pierro et al. [23] evaluated the radiochemical purity of ^{68}Ga-DOTANOC by means of TLC using two different supports, namely, iTLC-SG and Flash-TLC TecControl strips. The TLC results were validated by means of HPLC. The TLC mobile phase used for both methods was 0.1 M citrate/0.2 M HCl. No further details were provided.

The radiochemical purity of ^{68}Ga-DOTANOC was determined by Asti et al. [15] using 0.1 M sodium citrate mobile phase, pH 5 (method 1) and an RP-18F support, and by Mukherjee et al. [31, 32] and De Blois [33] et al., on an iTLC support. Both Asti and Mukherjee et al found that the Rf of DOTANOC was 0.0 and of free $^{68}Ga^{3+}$ was 0.9. It appears that this method only serves to detect free Ga^{3+}. They also used a second method which employed 1 M ammonium acetate/methanol (1:1) (method 2) as the mobile phase with an iTLC-SG support. For this method, Asti only stated that the Rf of DOTANOC was 0.9 and of

hydrolyzed ^{68}Ga was 0.1, without mentioning where free Ga^{3+} would migrate. This suggests that method 2 only serves to detect hydrolysed or colloidal ^{68}Ga, and method 1 would still be required to distinguish between labelled peptides and free gallium. De Blois et al. used a two strip method but do not comment on distinction between different impurities. In comparison, Mukherjee et al. stated that the Rf of ^{68}Ga-DOTA peptides was found to be 0.9 -1.0 and free Ga (III) and colloidal ^{68}Ga were both found to be 0.0 - 0.1 using method 2. This implies that method 2 can be used to distinguish the labelled peptides from both impurities, although it does not separate the impurities from each other. Mukherjee et al. also used paper chromatography (Whatman 3-MM) to determine radiochemical yield. Paper chromatography using 50 % aqueous acetonitrile revealed that ^{68}Ga-DOTANOC moved towards the solvent front and free Ga (III) and colloidal gallium both remained at the origin (Rf = 0.0). Mukherjee's results show that either of the methods can be used. iTLC gives better separation, is faster to run, less sample is required, there is better selectivity and different stationery phases can be selected. Paper chromatography may have been used because it is cheaper and suitable for the experiment.

Zhernosekov et al. [34] used TLC with a 0.1 M sodium citrate solution (pH 5) as the mobile phase on an aluminium backed silica gel 60 support to determine the radiochemical purity of ^{68}Ga-DOTATOC. The Rf of DOTATOC was 0.0 and that of free $^{68}Ga^{3+}$ was 0.9.

2.3.2.2 HPLC Methods

Di Pierro et al. [23] confirmed the results obtained with iTLC and Flash-TLC by means of an HPLC method that employed a Nucleosil C18 column (4 X 250mm) and a CH_3CN/H_20/0.1 % trifluoracetic acid (TFA) mobile phase. Zhernosekov et al [34] used an HPLC method with a 20 % acetonitrile/80 % trifluoroacetic acid/0.01 % water as the mobile phase with a Machery Nagel C18 column and De Blois [33] et al. confirmed their iTLC-SG results by means of HPLC using a Symmetry C18 column and 0.1 % (w/v) TFA (A) and methanol (B) mobile phases. Mukherjee et al. performed HPLC analysis by means of gradient elution using a C18 reversed phase column and water with 0.1 % TFA (solvent A) and acetonitrile with 0.1 % TFA (solvent B).

According to the European Pharmacopoeia draft monograph [21], the method for the radiochemical purity determination of ^{68}Ga-DOTATOC (edotreotide) by TLC on a silica gel plate uses a 77 g/L (1 M) solution of ammonium acetate in water and methanol (50:50 V/V) mobile phase. The monograph states that the Rf of ^{68}Ga in colloidal form is 0 - 0.1 and the Rf of ^{68}Ga-DOTATOC is 0.8-1.0. Not more than 3 % of the total radioactivity should be due to ^{68}Ga in colloidal form for TLC analysis. HPLC analysis should be performed with a mobile phase A consisting of TFA/water (0.1:99.9 V/V) and a mobile phase B mixture of TFA/Acetonitrile (0.1-99.9 V/V). The retention time of ^{68}Ga-DOTATOC is 4.2 minutes and $^{68}GaCl_3$ is 0.3 minutes. $^{68}GaCl_3$ should not exceed 2 % for HPLC analysis.

SUMMARY AND PROBLEM STATEMENT

From the literature review, it is clear that studies have been performed to compare different generator types, but no complete studies were carried out to evaluate all the quality parameters of the eluate over the entire life span of the generator (which could be for a period of 12-15 months). To determine the stability of the eluate it is important to determine how the yield, breakthrough, sterility and endotoxin levels change over the period of use. This information is vital especially for end users, who may not be in a position to perform these quality control tests and who can then be assured that the product quality remains stable. Moreover, the eluate is used to prepare ^{68}Ga-DOTA peptides to be administered for clinical use. The radiolabelled peptides must therefore meet strict pharmaceutical specifications, including high radiochemical purity. It is also important to validate the methods used to test the ^{68}Ga-labelled peptides to ensure that the results obtained are accurate and reliable. The literature is ambiguous regarding information provided by different TLC or iTLC methods. Although HPLC analysis is more accurate, iTLC is the preferred method because it is quicker and HPLC equipment is expensive and therefore not available in all facilities. It is however not clear if TLC or iTLC can be used without HPLC.

The aims of this study are therefore as follows:

Aim 1

To evaluate the ^{68}Ga eluate obtained from the iThemba LABS ^{68}Ge/^{68}Ga generator over a period of twelve months in order to ascertain whether the quality of the eluate remains stable over this time.

Aim 2

To compare iTLC and TLC methods, under various conditions, and to validate the accuracy of iTLC against HPLC.

Chapter 3: Methods, Materials and Equipment

This study was approved by the Stellenbosch University Research and Ethics committee (approval number: S15/07/143) and permission was granted for the experimental work to be conducted at iThemba LABS.

3.1 Preparation of Solutions

To prepare **0.6 M HCl**, 30% hydrochloric acid (HCl) Suprapur (9.642 M), Merck, catalogue number 1.00318.0025 was used. A volume of 63.41 ml of 30% HCl was used to prepare 1000 ml of 0.6 M HCl in ultra-pure water.

iTLC mobile phase 1: **0.1 M citrate buffer pH 5.0.** Tri-sodium citrate dihydrate supplied by Merck, catalogue number SAAR5822500EM, was used. A mass of 29.41 g of tri-sodium citrate dihydrate was weighed out and approximately 600 ml ultra-pure water was added to it. The pH of the solution was adjusted to 5.0 using 1N hydrochloric acid (HCl) and 1N sodium hydroxide (NaOH) and ultra-pure water was added to make up the final volume of 1 litre.

TLC mobile phase 2: **1 M ammonium acetate: methanol (1:1).** 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-Adrich) were added together immediately before use to make a 1:1 ammonium acetate : methanol solution.

HPLC mobile phase A: **0.1% (w/v) trifluoracetic acid (TFA)** solution was prepared using 0.67 ml of TFA Sigma-Aldrich, catalogue number 302031, per 1000 ml of ultra-pure water. **HPLC mobile phase B**: **Acetonitrile** with catalogue number 34851 from Sigma-Adrich was used.

To prepare **tryptic soy broth (TSB)**, 15 g of TSB powder (Sigma-Aldrich, catalogue number 22092) was dissolved in 500 ml of ultra-pure water. Preparation instructions were obtained from the container. The solution was heated with stirring and then boiled for 1 minute. After transfer to suitable storage containers it was sterilized by autoclaving at 121 °C for 15 minutes.

To prepare **thioglycolate broth (TB)**, 14.5 g of TB (Sigma-Aldrich, catalogue number 70157) was dissolved in 500 ml of ultra-pure water. The solution was boiled until the growth media was completely dissolved. Preparation instructions were obtained from the container. After transfer to suitable storage containers it was sterilized by autoclaving at 121 °C for 15 minutes.

The **reagents for the endotoxin testing** were prepared as per the instruction booklet provided with the Lonza chromogenic LAL test kit using LAL reagent water supplied in the kit. The Lonza LAL kit was obtained from Whitehead Scientific, catalogue number 50-647U.

1 ppm, 5 ppm and 10 ppm ICP standard solutions of zinc, iron, tin, copper, aluminium, titanium, gallium and germanium were prepared using 1000 ppm standard solutions obtained from Industrial Analytical (catalogue numbers: zinc:

88118, iron: 88073, tin: 88112, copper: 88061, aluminium: 33557, titanium: 35771, gallium: 88066 and germanium: 88067). The 1000 ppm standard solutions were diluted to 100 ppm with 0.6 M HCl before being used to prepare 1 ppm, 5 ppm and 10 ppm solutions of each metal.

All solutions were used for a period of 3 months.

3.2 Equipment

Gamma spectrometer	Genie 2000
Dose calibrator	Capintec CRC-55tR
Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES)	Jobin Yvonn Horiba Ultima
Radio-TLC scanner	Carroll Ramsey Associates
Incubator (32.5°)	Carbolite
Incubator (22.5°C)	Labcon
Microplate reader	ELx800
HPLC system:	
Binary HPLC Pump	Perkin Elmer Series S200 LC
HPLC InjectorRheodyne	model 7125
HPLC column	Phenomenex Luna C18 (250 X 4.6mm), 5µm
Integrator	Shimadzu Chromatopac CR8A

Radiation flow detector	Ortec NaI(Tl) detector with a high voltage power supply and rate meter

3.3 Elution of the Generators

Two 1850 MBq (50 mCi) ^{68}Ge/^{68}Ge generators were provided by iThemba LABS, South Africa. Generator 11/11/A(^{68}Ge)_02 will be referred to as generator A and Generator 12/15/A(^{68}Ge) will be referred to as generator B. The iThemba LABS generators were double loaded i.e. for a 1850 MBq generator 3700 MBq of Ge-68 was loaded onto the generator column and therefore the elution efficiency was greater than 100% at the start of the generator life. The nominal activity was used to calculate the elution efficiency, i.e. elution efficiency was expressed as a percentage of a single loaded activity value.

^{68}Ga eluates from the iThemba LABS produced ^{68}Ge/^{68}Ga generator were monitored over a 12 month period. During this time, the generators were eluted daily (excluding weekends) with 5 ml of 0.6 M hydrochloric acid (HCl). Quality control tests were repeated on a monthly basis. The activity of ^{68}Ga in the eluate was measured in the Capintec CRC-55tR Dose Calibrator.

3.4 Evaluation of Eluates

3.4.1 Determination of Yield

The elution efficiency (yield) was calculated as follows:

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

3.4.2 Determination of ^{68}Ge Breakthrough in eluates

After 24 hours, the eluted ^{68}Ga has decayed, and any ^{68}Ga present in the sample is only due to ^{68}Ge breakthrough. The ^{68}Ga present can therefore be used to calculate the amount of ^{68}Ge breakthrough present in an eluate. The ^{68}Ge breakthrough (radionuclidic purity) of a sample was measured at ≥ 24 hours after the elution of that sample using the Canberra gamma spectrometer with a germanium detector and Genie 2000 software. The ^{68}Ge in the samples was quantified using the 511 keV peak. Counts on the gamma spectrometer were performed for 1000 seconds each and spectra of the eluate, standard solution and background were obtained. The standard solution was prepared by diluting 1.11 MBq of ^{68}Ge solution (prepared at iThemba LABS) to a volume of 10 ml with 0.6 M HCl. The activity, date, time and volume were recorded on the label. The breakthrough was defined as the ratio of activity of ^{68}Ge over initial ^{68}Ga activity, expressed as a percentage. The ^{68}Ge breakthrough was calculated as follows:

$$^{68}\text{Ge after 24 hours} = \frac{\text{(Peak Area eluate – Peak Area Bkgrd) X Activity of Std}}{\text{(Peak Area Std – Peak Area Bkgrd)}}$$

Bkgrd = Background
Std = Standard

$$\text{Breakthrough (\%)} = \frac{^{68}\text{Ge activity x 100\%}}{^{68}\text{Ga activity at the time of elution}}$$

3.4.3 Determination of Metal Contaminants

The metal contaminants, i.e. gallium, germanium, zinc, iron, copper, tin, titanium and aluminium, were determined using a Jobin Yvon Horiba Ultima inductively coupled plasma optical emission spectrometer (ICP-OES). Calibration curves were obtained over a range of 1 ppm to 10 ppm and used to determine the concentration of these metals in the samples.

3.4.4 Sterility Testing

Sampling for sterility testing was performed aseptically in a biohazard cabinet in a clean room. The sterility testing of the ^{68}Ga eluate commenced on the day of elution using both tryptic soy broth (suitable for the culture of aerobic bacteria and fungi) and thioglycolate broth (suitable for the culture of anaerobic bacteria). In both instances a 5 ml volume of broth was added to 0.5 ml of ^{68}Ga eluate. The sample containing tryptic soy broth was incubated at 22.5 ± 2.5°C while the sample containing thioglycolate broth was incubated at 32.5 ± 2.5°C [27]. An *E. Coli* positive and one negative control were incubated along with the sample for two weeks and checked on a daily basis for appearance of growth. The change in

appearance of the media from clear to cloudy was regarded as an indicator for bacterial growth.

3.4.5 Endotoxin Testing

Endotoxin testing was performed using the chromogenic method. A Lonza kit containing the endotoxin stock solution, LAL reagent, substrate and LAL reagent water was used. The endotoxin stock solution was used to prepare a set of endotoxin standards, ranging from 0.1 endotoxin units per millilitre (EU/ml) to 1.0 EU/ml. The absorbance which was determined using a BioTek ELx800 plate reader was plotted against the concentration of the standards to create a calibration curve. The calibration curve was then used to determine the concentration of endotoxins in the eluates. The endotoxin tests were performed on the day of elution. The maximum dilution volume was determined to be 10 and the pH of samples was adjusted to within the required pH range of 6.0 – 8.0 with 1 N sodium hydroxide prepared with LAL reagent water.

3.5 Quality Control of ^{68}Ga-labelled peptides

Various quality control procedures such as high performance liquid chromatography (HPLC) and instant thin layer chromatography (iTLC) were used to determine the radiochemical purity of the labelled peptides.

3.5.1 HPLC

The HPLC analysis was performed with a Phenomenex Luna C18 (250 X 4.6 mm, 5 µm) analytical column using gradient elution with 0.1 % TFA in water (mobile phase A) and 100 % acetonitrile (mobile phase B) [16]. The HPLC program was as

follows: 0–2 min 100 % A, 2-12 min 100 % A to 30 % A and 70 % B, 12- 15 min 30 % A and 70 % B to 100 % B, 15 -20 min 100 % B. Under these conditions the free ^{68}Ga eluted within a retention time range of 2.764 to 2.933 minutes.

3.5.2 TLC

Two TLC methods were investigated. Method one used 0.1 M citrate buffer, pH 5 (mobile phase 1) as the developing mobile phase and silica gel impregnated instant thin layer chromatography medium (iTLC SGI0001) purchased from SMM Instruments as a solid support. Method 2 made use of 1 M ammonium acetate/methanol (1:1) as mobile phase (mobile phase 2) with either Silica Gel 60 TLC sheets (Merck) or iTLC-SG as the stationary phase. For both methods the strip was 9 cm long, 1.5 cm wide and the spot with activity (185 to 370 MBq) was placed at 1.5 cm from the bottom of the strip. The TLC or iTLC strip was placed in a chromatography tank containing 10 ml of the mobile phase and allowed to develop until the solvent front reached a pre-marked spot, applied with a highlighter pen, 0.5 cm from the top of the strip. The contact of the front with this spot caused a blotting effect which facilitated its visualisation. Thereafter the strip was removed from the tank and allowed to air dry.

The dry strips were scanned using a Carroll Ramsay Associates radio-TLC scanner, coupled with a Shimadzu Chromatopak CR8A. Chromatograms were printed out and displayed peaks with retention times (Rt) in minutes. The retention times were subsequently converted to the retention factor (Rf) values. The distance migrated was determined by multiplying the Rt obtained by the scan rate (2 cm/min) of the radio-TLC scanner. However, due to the geometry of the TLC scanner, the retention time included a dead distance (which is the distance from

the start of scanning to the origin of the plate or strip). The dead distance was always a fixed parameter and had to be determined. Therefore, activity was applied to the origin of a strip and scanned without developing it in the chromatography tank. The Rt obtained for the activity spot at the origin multiplied by the scan rate of the radio-TLC scanner was the dead distance. To convert the Rt of the developed species to distance it was multiplied by the scan rate of the radio-TLC scanner and the dead distance was subtracted from the result to obtain the real distance. Rf values were calculated by dividing this result by 7.2 cm, which was the distance in cm between the origin and the solvent front. See figure 2.

The iTLC methods were validated by comparing their results with HPLC results obtained for the same sample.

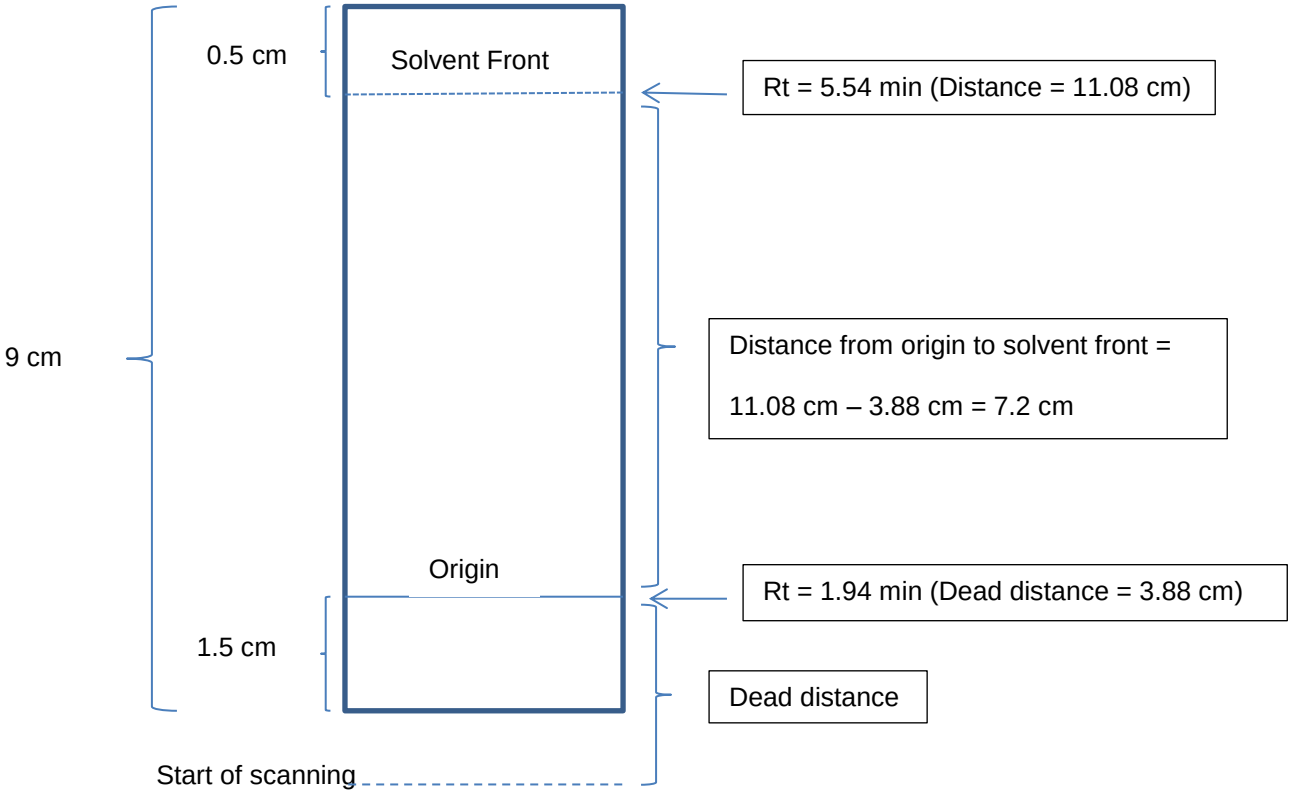

Figure 2: Conversion of retention time (Rt) on iTLC chromatogram to distance. Activity was spotted at the origin and the plate was scanned without developing it in mobile phase. The scan rate of the radio-TLC scanner is 2 cm/min. The dead distance is the distance from the start of scanning to the origin = Rt at the origin X scan rate. Distance = (Rt X scan rate) – dead distance.

3.5.2.1 Determination of detectable amount of $GaCl_3$

iTLC was carried out on labelled peptide solutions that had been spiked with 2 % $GaCl_3$. The purpose of this experiment was to verify that a clear separation between $^{68}GaCl_3$ and ^{68}Ga labelled peptides was obtained and that the maximum amount of allowable $^{68}GaCl_3$ could be detected by means of iTLC. The spiked mixtures were prepared as follows: The activity of the purified ^{68}Ga-DOTA peptide was measured in the Capintec dose calibrator and an amount of $^{68}GaCl_3$ that was equivalent to 2 % of the total activity was added. The $^{68}GaCl_3$ was measured in the Capintec Dose Calibrator and the time of the measurement was recorded. The same was done with the labelled peptide before adding the $^{68}GaCl_3$. The measured activities were corrected for decay to determine the exact activity of the $^{68}GaCl_3$ and labelled peptide in the mixture. The mixture was spotted on iTLC medium and developed in mobile phase 1. The results obtained were verified by means of HPLC.

3.5.2.2 Comparison using dried and undried iTLC paper

iTLC strips were dried at 80 °C for 2 hours on the day of the experiments. The dried paper was stored in a sealed bag inside a dessicator until used. A dried and an undried iTLC plate were spotted with the same sample of ^{68}Ga-DOTATATE. This procedure was repeated five times using different batches of ^{68}Ga-DOTATATE. Five experiments were also performed using ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC. The iTLC plates were developed in mobile phase 1 and scanned on the radio-TLC scanner. After scanning, the developed strips containing free $^{68}GaCl_3$ and labelled peptide were also cut in segments of 1 cm each (measured from the origin) and the activity on each segment measured in a Capintec dose

calibrator. This showed the distribution of free $^{68}GaCl_3$ and labelled peptide after chromatography.

3.5.2.3 Colloids

To induce the formation of colloids, the pH of the labelling mixture was adjusted to pH 5.0 by adding sodium acetate buffer. Samples of peptide labelled at this higher pH were analysed by iTLC using mobile phase 1, iTLC using mobile phase 2 and by means of HPLC. Radiolabelling was also performed at the normal labelling pH of 3.5 – 4.0 and the results were compared to those obtained at pH 5.0. With mobile phase 1, $^{68}GaCl_3$ is expected to migrate to the solvent front while ^{68}Ga-labelled peptides remain at the origin. Colloids are not separated from the ^{68}Ga-labelled peptides with this method. With mobile phase 2, ^{68}Ga impurities are expected to remain at the origin while the ^{68}Ga-labelled peptides migrate to the solvent front. With the HPLC method only $^{68}GaCl_3$ and ^{68}Ga-labelled peptides are separated, while colloids will be retained at the beginning of the column or pre-column.

3.6 Data analysis

To visualize the change in performance of $^{68}Ge/^{68}Ga$ generators over time the data collected are depicted in graphs i.e.

- Percentage ^{68}Ga breakthrough vs. time
- Percentage yield vs. time
- Concentration of metal contaminants vs. time
- Endotoxin concentration vs. time

Since this data is expected to change over time, only trends were observed. All results were compared to pharmacopoeial limits and results obtained from two different generators were compared. The results from different TLC methods and the HPLC method used to determine radiochemical purity of the labelled product were compared by visual inspection. The Paired T-test was used to determine if there was a significant difference in the results obtained for experiments performed using the dried versus undried stationary phase for iTLC.

Chapter 4: Results

4.1 Generator Eluate

Generator A and generator B were stored in a biohazard cabinet within a clean room and were eluted over a period of 360 days (12 months). The generators were eluted manually on a daily basis and the ^{68}Ga yield, ^{68}Ge breakthrough, metal contaminants, sterility and endotoxin concentration were determined using the eluate that was collected every thirty days.

The ^{68}Ga yield of generator A was 132.0 % initially and gradually decreased to 87.8 % after 12 months. The ^{68}Ga yield of generator B was 125.0 % initially and progressively decreased to 85.0 % after 12 months (Figure 3).

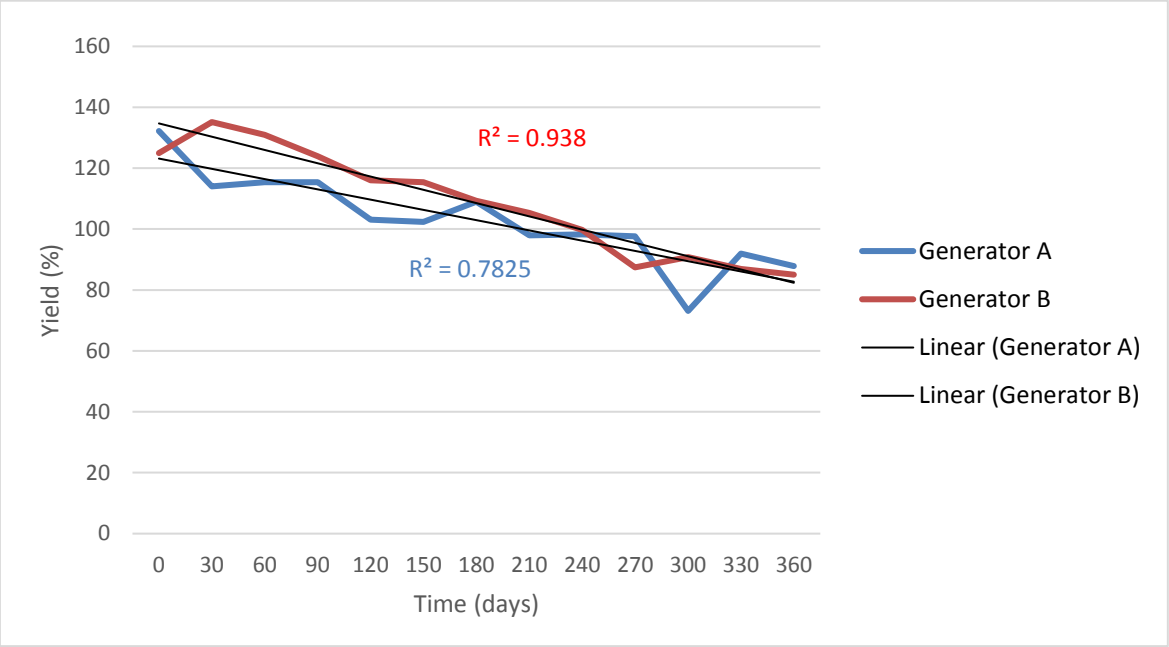

Figure 3: ^{68}Ga yields obtained for generator A and B over a 12 month period

Eluates were measured in the Capintec dose calibrator and the measurements were used to calculate the yield. Measurements were done at 30 day intervals (Correlation coefficient for generator A was 0.7825 and for generator B was 0.938).

The ^{68}Ge breakthrough increased from 0.0003 % at the start of use to 0.1560 % after 12 months in generator A. The ^{68}Ge breakthrough increased from 0.0004 % to 0.2672 % in generator B. A spike was observed in both generators at 300 days (Figure 4). This coincided with an elution after a period of 25 days during which the generator had not been eluted.

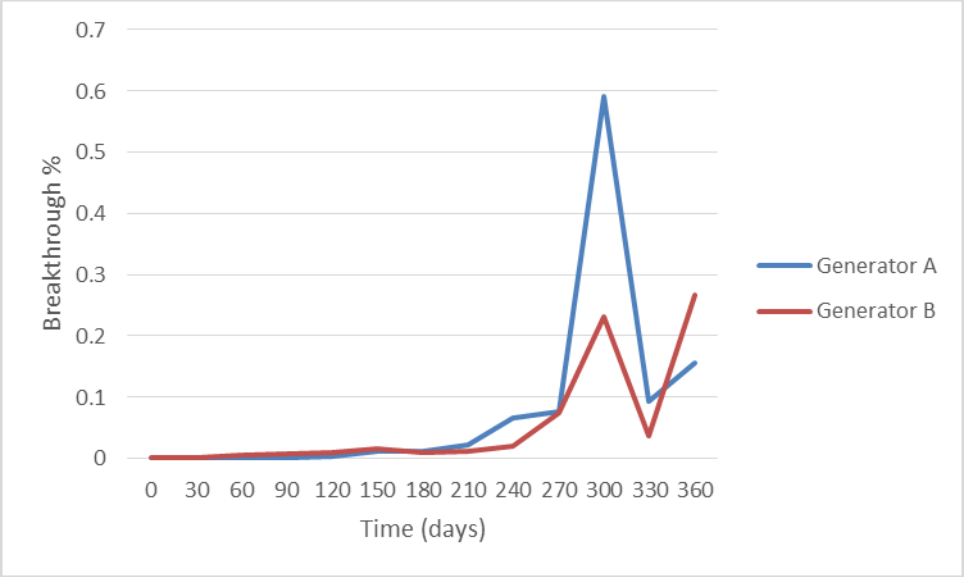

Figure 4: ^{68}Ge breakthrough for generator A and B over a 12 month period

The breakthrough was determined every 30 days using a gamma spectrometer at least 24 hours after elution.

Metal contaminants were determined by means of ICP-OES every 30 days and are recorded in Table 3 and 4 and Figure 5. The total metal contaminants fluctuated slightly but Table 3 and 4 show that the average metal contaminants over the year remained below the pharmacopeial limit of 10 ppm throughout the year for both generators, except at 360 days for generator B, where it increased to 13.48 ppm (see Table 4). The zinc concentration was 10.15 ppm and was probably due to a period of non-elution and the time between elution and analysis during which the ^{68}Ga decayed to ^{68}Zn.

TABLE 3: Metal Contaminant Data: Generator A

ICP-OES analysis was performed on eluates from generator A at 30 day intervals.

No. of Days	Metal Contaminants (ppm)								
	Zn	Fe	Cu	Sn	Ti	Al	Ge	Ga	Total Metals
0	1.38	0.12	0.00	1.12	0.00	3.01	0.27	0.13	6.03
30	1.96	0.11	0.03	0.19	0.10	0.12	0.47	0.17	3.15
60	4.69	0.04	0.00	0.36	3.61	0.00	0.65	0.00	9.35
90	4.42	0.25	0.01	0.98	0.08	0.98	0.46	0.15	7.33
120	1.17	0.04	0.00	0.35	3.61	0.00	0.64	0.00	5.81
150	0.31	0.15	0.03	0.23	0.00	1.23	0.53	0.11	2.59
180	1.38	0.12	0.06	1.12	0.71	3.01	0.27	0.13	6.80
210	0.35	0.12	0.03	0.18	0.10	0.15	0.43	0.18	1.54
240	1.98	0.04	0.00	1.17	0.00	1.07	0.03	0.11	4.40
270	0.45	0.08	0.04	0.97	0.02	1.12	0.56	0.05	3.29
300	2.21	0.08	0.05	0.89	0.02	1.34	0.40	0.05	5.04
330	0.46	0.08	0.04	0.97	0.02	1.12	0.56	0.05	3.30
360	3.54	0.11	0.01	1.36	0.02	0.65	0.77	0.11	6.57
Average	1.87	0.10	0.02	0.76	0.64	1.06	0.46	0.10	5.02
STDEV	1.50	0.06	0.02	0.43	1.33	0.99	0.19	0.06	2.22

TABLE 4: Metal Contaminant Data: Generator B

ICP-OES analysis was performed on eluates from generator B at 30 day intervals.

No. of Days	Metal Contaminants (ppm)								
	Zn	Fe	Cu	Sn	Ti	Al	Ge	Ga	Total Metals
0	1.93	0.07	0.02	0.22	0.01	0.29	0.21	0.75	3.50
30	4.55	0.06	0.00	0.95	0.00	0.83	0.17	0.1	6.66
60	4.34	0.20	0.07	0.70	0.00	0.91	0.05	0.01	6.28
90	3.28	0.23	0.17	0.80	0.08	0.65	0.05	1.01	6.27
120	6.62	0.17	0.07	0.81	0.00	1.08	0.04	0.02	8.81
150	2.73	0.05	0.00	0.97	0.00	0.48	0.17	0.12	4.52
180	3.27	0.34	0.18	1.31	0.16	0.75	0.12	0.64	6.77
210	6.14	0.16	0.00	2.4	0.05	0.47	0.30	0.07	9.59
240	7.56	0.18	0.07	0.93	0.00	0.81	0.04	0.01	9.60
270	5.87	0.19	0.01	0.65	0.08	1.14	0.15	0.46	8.55
300	2.48	0.00	0.00	1.05	0.00	0.94	0.14	0.00	4.61
330	3.58	0.10	0.01	0.68	0.02	0.38	0.76	0.10	5.63
360	10.15	0.42	0.38	0.31	0.40	1.01	0.41	0.40	13.48
Average	4.81	0.17	0.08	0.91	0.06	0.75	0.20	0.28	7.25
STDEV	2.36	0.12	0.11	0.53	0.11	0.28	0.20	0.34	2.70

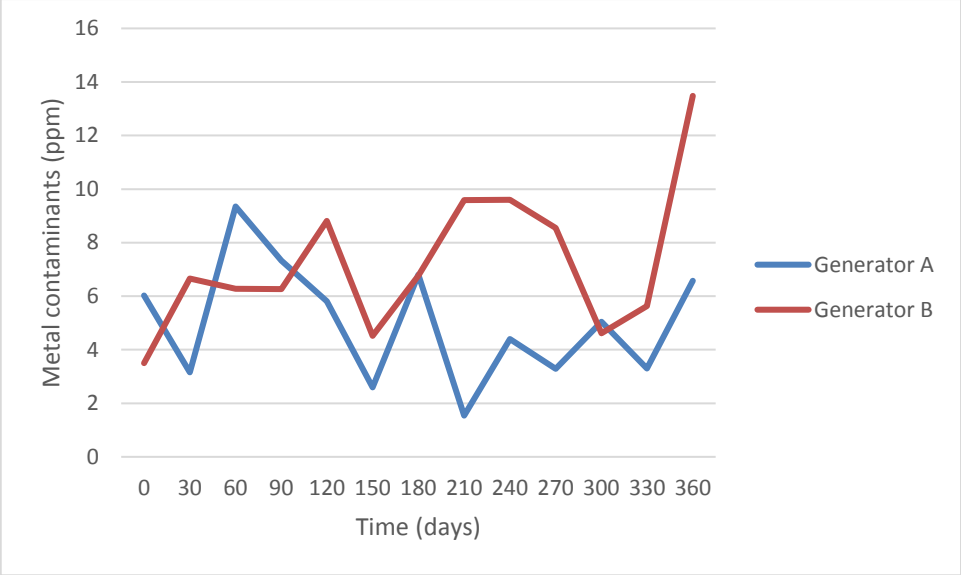

Figure 5: Total metal contaminants for generator A and B over a 12 month period

The concentration of metals in the eluates of generator A and B was determined using a Jobin Yvon ICP-OES at 30 day intervals.

Sterility and Endotoxins

No visible bacterial or fungal growth was seen in any of the generator eluates after 14 days of incubation. The endotoxin concentration in the ^{68}Ga eluates from generator A and B are shown in Figure 6. Endotoxin concentrations remained below 2 EU/ml and were within the pharmacopoeial limit of 175 EU/ml for the duration of the 12-month period.

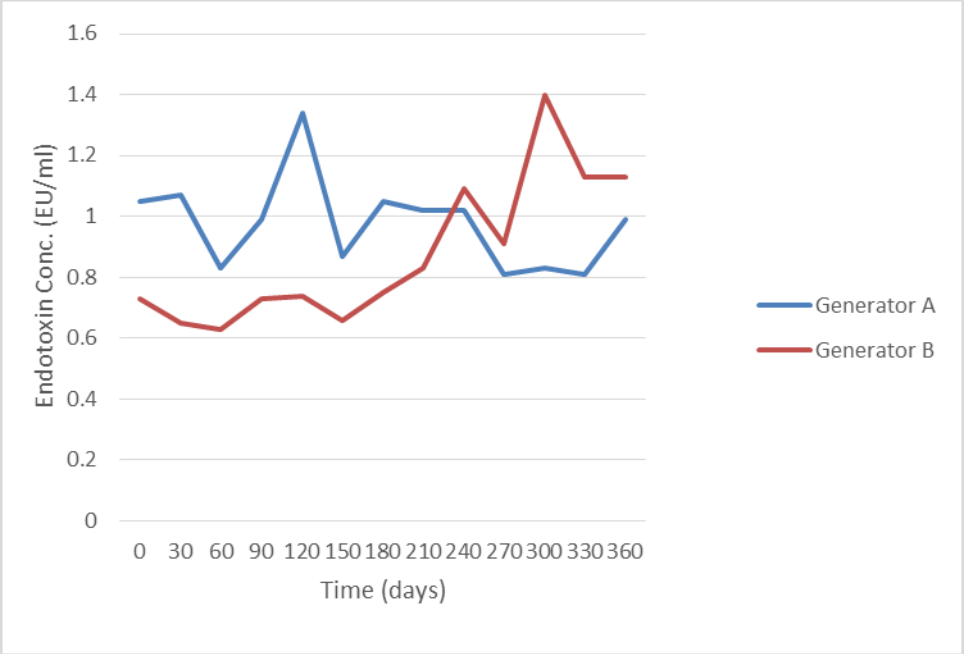

Figure 6: Endotoxin Concentration for generator A and B over a 12 month period: The endotoxin concentration in the eluates of generator A and B was determined using a chromogenic LAL kit every 30 days.

4.2 Analysis of ^{68}Ga-labelled peptides

4.2.1 HPLC Analysis

HPLC analysis was performed on $^{68}GaCl_3$ and ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC samples with 0.1 % TFA and 100 % acetonitrile mobile phases. The retention time (Rt) of $^{68}GaCl_3$ ranged from 2.764 minutes to 2.993 minutes, the Rt of ^{68}Ga-DOTATATE was between 12.077 minutes and 12.183 minutes, ^{68}Ga-DOTATOC was between 12.087 minutes and 12.250 minutes and ^{68}Ga-DOTANOC was between 12.607 minutes and 12.809 minutes (Table 5). The chromatograms for all the ^{68}Ga-labelled peptides showed sharp peaks with a clear separation between $^{68}GaCl_3$ and ^{68}Ga-labelled peptides observed. An example of a typical chromatogram is shown in Figure 7. The retention times of the three

peptide peaks are very close to each other but this does not pose a problem because only one peptide is labelled at a time.

TABLE 5: HPLC Retention time (Rt) of $^{68}GaCl_3$, ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC

$^{68}GaCl_3$ and ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC were analysed by means of HPLC using HPLC using mobile phase A: 0.1% trifluoracetic acid (TFA) solution and mobile phase B: 100% acetonitrile (0–2 min 100 % A, 2-12 min 100 % A to 30 % A and 70 % B, 12- 15 min 30 % A and 70 % B to 100 % B, 15 -20 min 100 % B.

Exp. No.	Rt $^{68}GaCl_3$ (min.)	Rt ^{68}Ga-'TATE (min.)	Rt ^{68}Ga-'TOC (min.)	Rt ^{68}Ga-'NOC (min.)
1	2.933	12.166	12.117	12.808
2	2.764	12.124	12.087	12.607
3	2.917	12.183	12.217	12.737
4	2.865	12.087	12.200	12.733
5	2.877	12.077	12.292	12.809
6	2.931	12.095	12.116	12.823
7	2.941	12.098	12.250	12.763
8	2.981	12.167	12.133	12.802
Average	2.901	12.125	12.178	12.760
SDEV	0.066	0.042	0.074	0.071

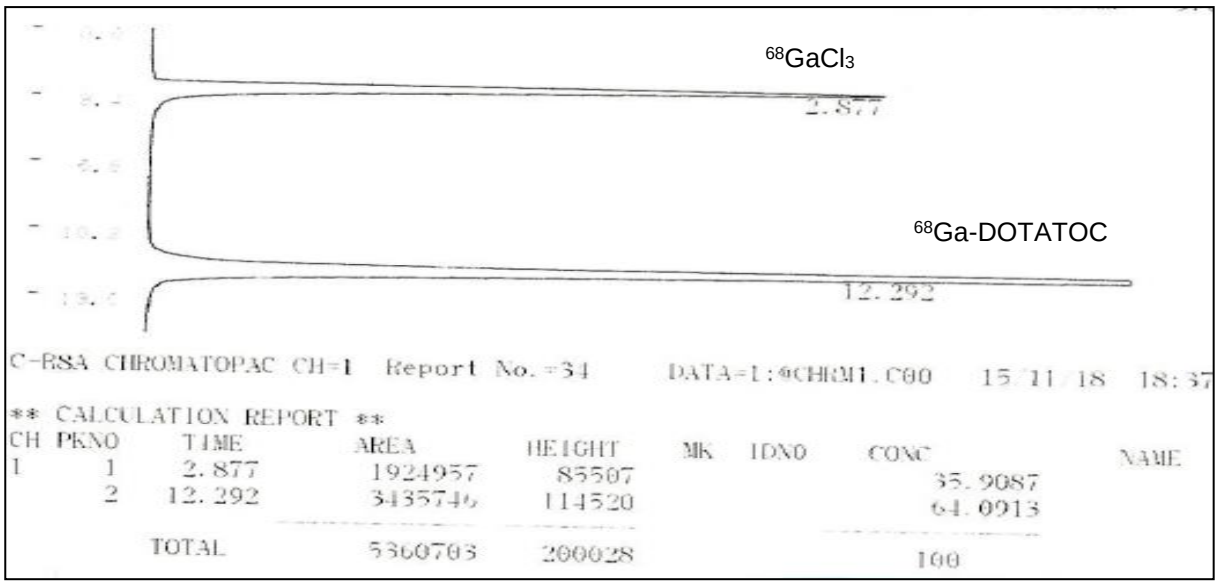

Figure 7: Typical HPLC chromatogram of ^{68}Ga-DOTATOC before Sep-Pak purification

4.2.2 iTLC Analysis

4.2.2.1 iTLC analysis on undried and dried strips using mobile phase 1 (0.1 M citrate buffer, pH 5)

iTLC analysis was performed on $^{68}GaCl_3$ and ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC samples using undried iTLC strips, and strips that had been dried at 80 °C prior to use. The average retention factor (Rf) for $^{68}GaCl_3$ was found to be 0.99 and 0.98 on undried and dried iTLC strips respectively (Table 6). The average Rf for ^{68}Ga-DOTATATE was found to be 0.12 on undried iTLC strips and 0.15 on dried iTLC strips. The average Rf of ^{68}Ga-DOTATOC was 0.07 on undried iTLC strips and 0.08 on dried iTLC strips. The average Rf of ^{68}Ga-DOTANOC was 0.13 on undried iTLC strips and 0.10 on dried iTLC strips (Table 6). Separation was achieved between the $^{68}GaCl_3$ peak and ^{68}Ga-peptide peaks. An example of typical chromatogram is shown in Figure 8.

TABLE 6: iTLC analysis of $^{68}GaCl_3$ ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC using undried and dried iTLC strips

$^{68}GaCl_3$, ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC were spotted on undried and dried iTLC strips and the strips were developed in 0.1 M citrate buffer, pH 5. The developed strips were scanned on a radio-TLC scanner.

	Rf values on iTLC medium							
Exp No.	$^{68}GaCl_3$		^{68}Ga-DOTATATE		^{68}Ga-DOTATOC		^{68}Ga-DOTANOC	
	Undried iTLC	Dried iTLC	Undried iTLC	Dried iTLC	Undried iTLC	Dried iTLC	Undried iTLC	Dried iTLC
1	0.98	0.97	0.12	0.13	0.07	0.06	0.12	0.11
2	0.98	0.98	0.13	0.14	0.06	0.08	0.13	0.10
3	1.00	1.00	0.11	0.16	0.08	0.09	0.13	0.10
4	1.00	0.99	0.10	0.15	0.07	0.07	0.13	0.11
5	0.99	0.97	0.13	0.16	0.07	0.09	0.11	0.09
AVG	0.99	0.98	0.12	0.15	0.07	0.08	0.13	0.10
SDEV	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	p = 0.0993		p = 0.0285 *		p = 0.2420		p = 0.0042 *	

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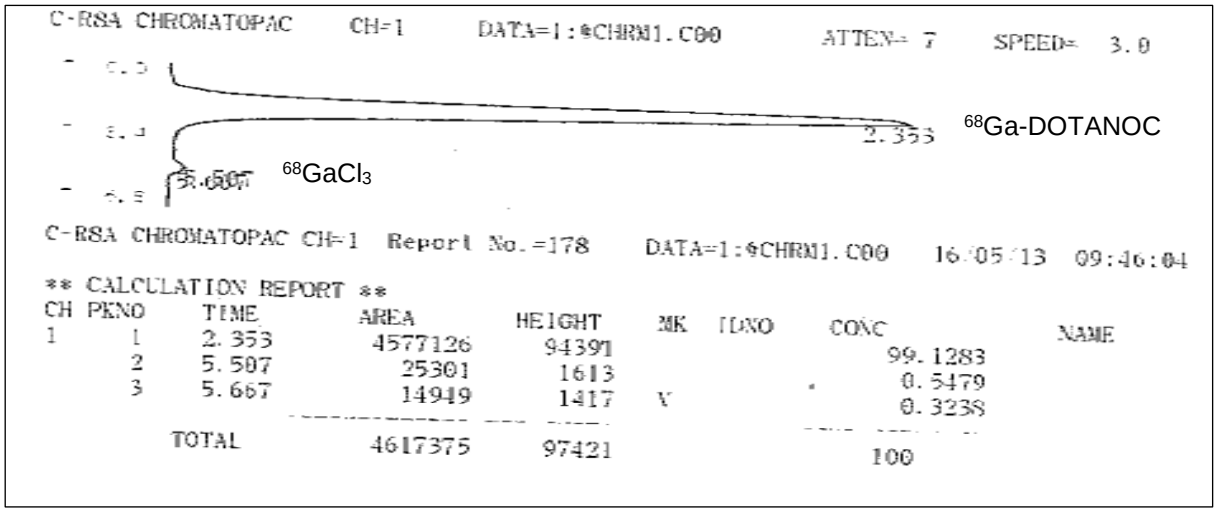


Figure 8: Typical iTLC chromatogram of ^{68}Ga-DOTANOC before Sep-Pak purification using 0.1 M citrate buffer, pH 5

The following tables show the results of counting 1 cm segments of the iTLC strips in a dose calibrator. The activity in each segment is expressed as percentage of the activity on the entire strip. This experiment was only done using mobile phase 1 (0.1 M sodium citrate). For the strips spotted with $^{68}GaCl_3$, 100 % of the activity was found on segment 9, both on undried and dried iTLC strips. The results were therefore not tabulated. For the iTLC strips spotted with ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC the results show that most of the activity was in the first 5 segments. The data are provided in Tables 7, 8 and 9.

TABLE 7: Percentage ^{68}Ga-DOTATATE on dried and undried iTLC strip segments

^{68}Ga-DOTATATE was spotted on iTLC strips, developed in 0.1 M citrate buffer, pH 5 and then cut into 1cm segments. Each segment was measured in the Capintec dose calibrator.

Segment No	% ^{68}Ga-DOTATE on undried iTLC Paper						% ^{68}Ga-DOTATATE on dried iTLC Paper					
	Experiment number						Experiment number					
	1	2	3	4	5	AVG	1	2	3	4	5	AVG
1	2.0	2.1	1.0	0.0	0.0	1.0	0.0	0.0	0.6	0.0	0.0	0.1
2	16.3	19.7	16.9	14.6	12.6	16.0	21.1	18.8	21.0	11.4	23.2	19.1
3	32.0	19.2	15.0	9.4	15.2	18.2	54.0	37.9	36.2	41.8	46.8	43.3
4	36.2	35.0	45.0	47.1	48.3	42.3	15.0	31.3	30.0	32.3	19.8	25.7
5	5.8	9.8	12.9	19.5	13.8	12.4	3.3	4.9	5.3	6.1	3.4	4.6
6	2.4	4.3	3.5	3.9	3.7	3.6	1.3	2.8	2.6	2.8	1.6	2.2
7	2.0	2.6	2.5	2.6	1.5	2.2	1.7	1.7	1.8	2.0	1.6	1.7
8	1.6	3.9	0.8	1.3	2.2	2.0	1.3	0.9	1.2	1.4	0.9	1.2
9	1.6	3.4	2.5	1.6	2.6	2.3	2.2	1.7	1.4	2.2	2.8	2.0

TABLE 8: Percentage ^{68}Ga-DOTATOC on dried and undried iTLC strip segments

^{68}Ga-DOTATOC was spotted on iTLC strips, developed in 0.1 M citrate buffer, pH 5 and then cut into 1cm segments. Each segment was measured in the Capintec dose calibrator.

Segment No	% ^{68}Ga-DOTATOC on undried iTLC Paper						% ^{68}Ga-DOTATOC on dried iTLC Paper					
	Experiment number						Experiment number					
	1	2	3	4	5	AVG	1	2	3	4	5	AVG
1	1.7	1.0	1.2	3.1	3.0	2.0	0.6	1.3	3.1	5.1	3.3	2.7
2	29.9	38.4	25.7	28.6	31.1	30.7	32.6	34.0	55.9	30.1	25.4	35.6
3	52.7	48.2	48.2	31.9	35.3	43.2	55.5	54.2	33.5	32.6	33.6	41.9
4	11.1	7.6	17.4	18.0	15.6	13.9	7.2	6.6	2.9	12.1	16.2	9.0
5	1.2	1.5	3.4	7.6	5.6	3.8	0.9	0.9	1.1	4.5	4.9	2.5
6	1.0	1.0	1.7	2.3	1.9	1.6	0.5	0.6	0.9	3.0	3.9	1.8
7	0.8	1.0	1.2	2.3	1.9	1.4	0.5	0.9	0.5	2.7	2.8	1.5
8	1.2	1.0	0.5	2.5	1.7	1.4	1.1	0.9	1.1	4.7	4.1	2.4
9	0.5	0.5	0.7	3.9	3.9	1.9	1.1	0.9	1.1	5.1	5.9	2.8

TABLE 9: Percentage ^{68}Ga-DOTANOC on dried and undried iTLC strip segments

^{68}Ga-DOTANOC was spotted on iTLC strips, developed in 0.1 M citrate buffer, pH 5 and then cut into 1cm segments. Each segment was measured in the Capintec dose calibrator.

Segment No	% ^{68}Ga-DOTANOC on undried iTLC Paper						% ^{68}Ga-DOTANOC on dried iTLC Paper					
	Experiment number						Experiment number					
	1	2	3	4	5	AVG	1	2	3	4	5	AVG
1	2.1	1.5	5.9	5.8	4.6	4.0	2.2	2.3	2.1	4.1	4.1	2.9
2	14.8	15.7	17.8	67.1	52.3	33.5	28.2	17.6	17.5	37.0	33.6	26.8
3	34.2	52.8	47.9	15.2	29.6	35.9	51.0	44.1	40.9	41.1	41.3	43.7
4	31.0	17.8	14.8	2.0	2.0	13.5	12.5	30.7	32.8	5.6	5.6	17.4
5	6.3	4.1	3.0	1.3	1.3	3.2	1.5	2.0	2.9	3.1	3.0	2.5
6	2.5	1.0	4.7	1.6	1.5	2.3	1.2	1.0	1.8	2.2	2.7	1.8
7	2.1	2.0	2.4	1.3	1.3	1.8	0.5	0.8	0.5	1.6	2.4	1.2
8	3.2	2.5	1.8	1.6	2.0	2.2	1.5	0.8	0.5	2.1	3.0	1.6
9	3.9	2.5	1.8	4.0	5.4	3.5	1.5	0.8	1.0	3.2	4.4	2.2

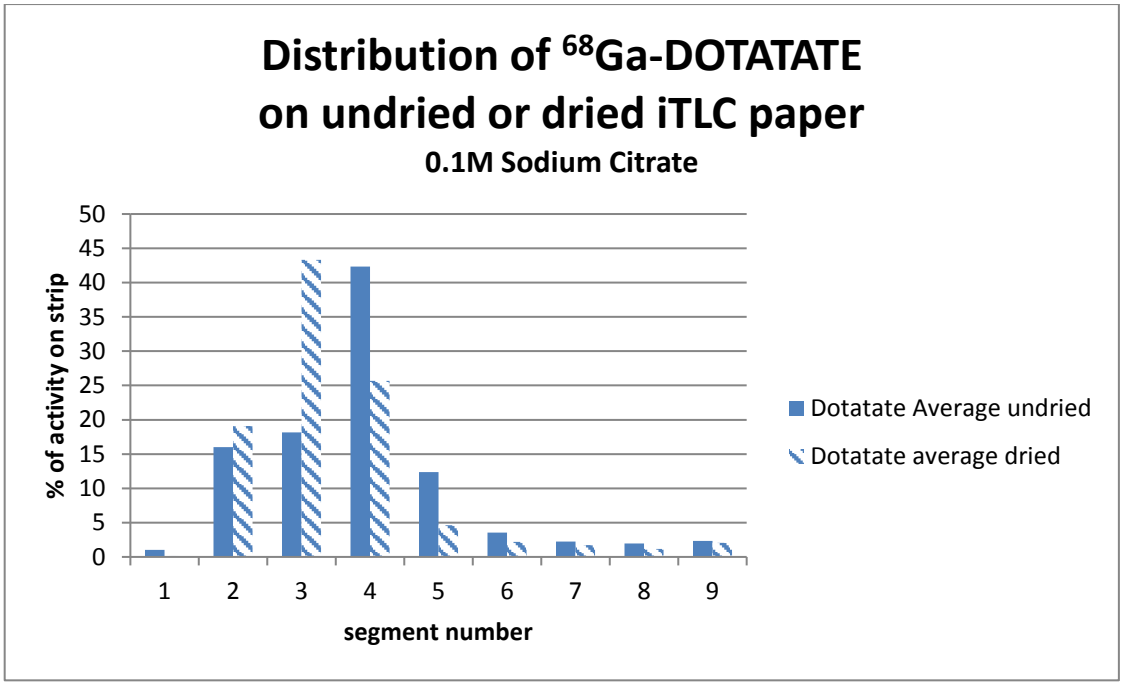


Figure 9: Distribution of ^{68}Ga-DOTATATE on undried and dried iTLC paper

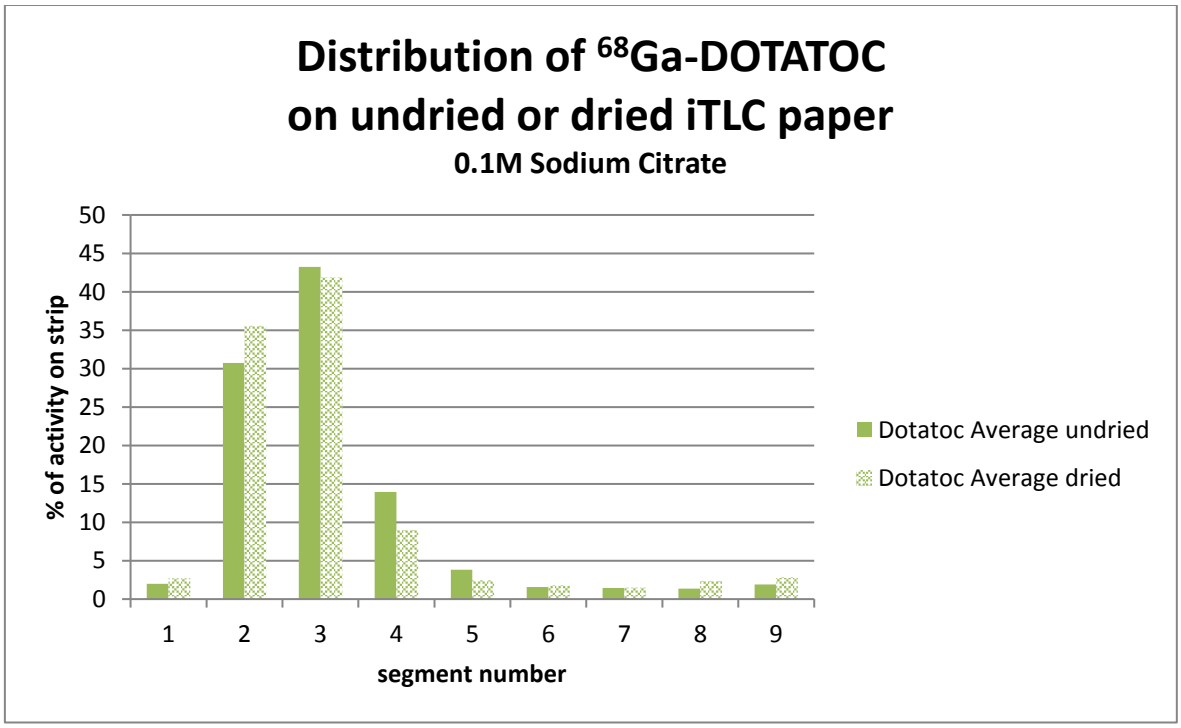


Figure 10: Distribution of ^{68}Ga-DOTATOC on undried and dried iTLC paper

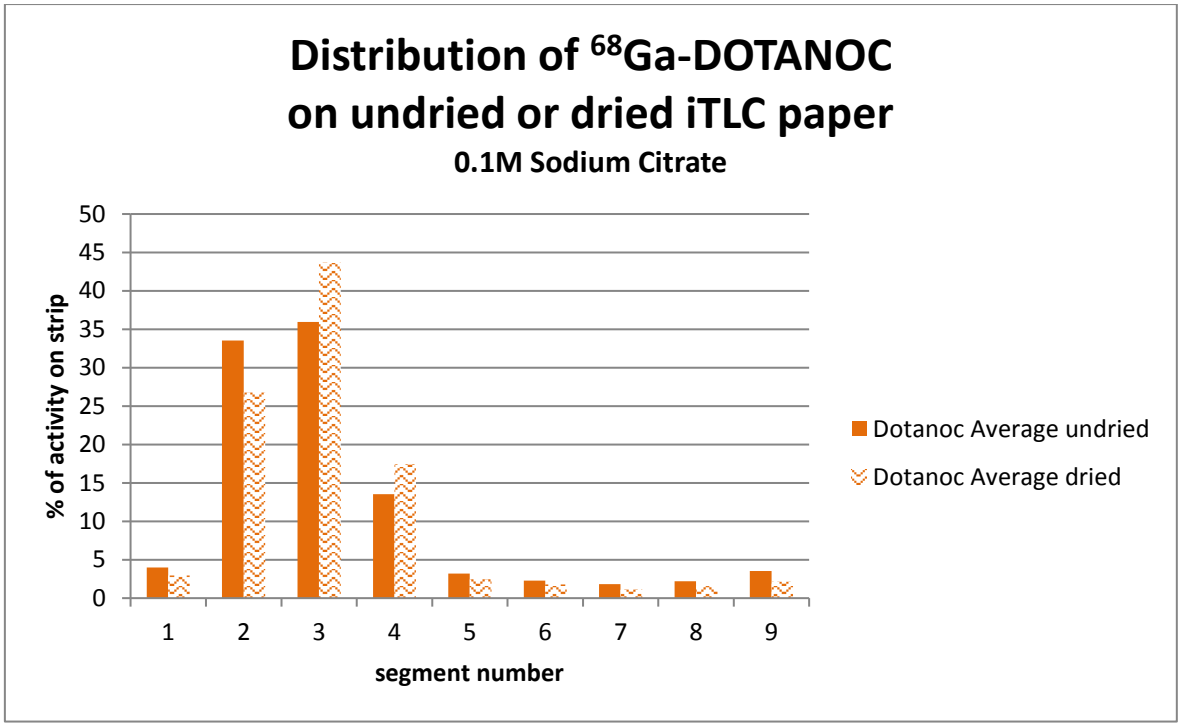


Figure 11: Distribution of ^{68}Ga-DOTANOC on undried and dried iTLC paper

Figures 9, 10 and 11, show that most of the labelled peptide is distributed in segments 2 to 4, on both dried and undried iTLC paper. The paired-T test proved that the results on dried and undried iTLC paper did not differ significantly for ^{68}Ga-DOTATOC but a significant difference was found between the results on dried and undried iTLC paper for ^{68}Ga-DOTATATE and ^{68}Ga-DOTANOC.

4.2.2.2 Determination of the detectable amount of $^{68}GaCl_3$

Purified, labelled peptides were spiked with 2 % of $^{68}GaCl_3$ and the samples were analysed by means of both HPLC and iTLC (undried). The results obtained were compared. Table 10 shows that the detected amount of $^{68}GaCl_3$ was found to be higher using HPLC than iTLC i.e. 3.44 % (HPLC) and 0.50 % (iTLC) for ^{68}Ga-DOTATATE, 2.85 % (HPLC) and 1.00 % (iTLC) for ^{68}Ga-DOTATOC and 3.86 % (HPLC) and 0.97 % (iTLC) for ^{68}Ga-DOTANOC.

TABLE 10: Radiochemical purities of ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC after Sep-Pak purification using iTLC and HPLC

Purified ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC was spiked with 2 % of $^{68}GaCl_3$, and the radiochemical purity was determined with iTLC (using undried iTLC paper and 0.1 M citrate buffer, pH 5), and HPLC

Experiment No.	Peptide	HPLC		iTLC	
		% $^{68}GaCl_3$	% Peptide	% $^{68}GaCl_3$	% Peptide
1	^{68}Ga-DOTATATE	3.25	96.75	0.97	99.03
2		3.28	96.72	0.95	99.05
3		3.22	96.78	0.34	99.66
4		3.63	96.37	0.22	99.78
5		3.83	96.17	0.01	99.99
AVG		3.44	96.56	0.50	99.50
STDEV		0.27	0.27	0.44	0.44
6	^{68}Ga-DOTATOC	2.72	97.28	0.59	99.41
7		2.56	97.44	1.42	98.58
8		3.36	96.64	1.04	98.96
9		3.10	96.91	1.01	98.99
10		2.50	97.50	0.92	99.08
AVG		2.85	97.15	1.00	99.00
STDEV		0.37	0.37	0.30	0.30
11	^{68}Ga-DOTANOC	4.46	95.54	0.87	99.13
12		4.38	95.62	0.64	99.36
13		2.99	97.01	1.30	98.70
15		3.20	96.80	1.08	98.92
15		3.38	96.62	0.96	99.04
AVG		3.68	96.32	0.97	99.03
STDEV		0.69	0.69	0.24	0.24

4.2.2.3 TLC analysis using mobile phase 2 (1 M ammonium acetate: methanol (1:1))

TLC analysis was also performed on unpurified samples using mobile phase 2 (1 M ammonium acetate: methanol (1:1)) and iTLC plates. The chromatogram, peaks obtained with this method were sharper than the peaks obtained using mobile phase 1 but the peaks overlapped slightly. The average of Rf for $^{68}GaCl_3$ was 0.15, ^{68}Ga-DOTATATE was 0.46, ^{68}Ga-DOTATOC was 0.52 and ^{68}Ga-DOTANOC was 0.44. The results are displayed in Table 11. An example of a typical chromatogram is shown in Figure 12.

TABLE 11: Retention factor (Rf) of $^{68}GaCl_3$, ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC on silica gel plates, 1 M ammonium acetate: methanol (1:1) mobile phase

Retention factors (Rf) of $^{68}GaCl_3$ and ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC were determined using mobile phase 2 and the developed strips were scanned strip on a radio-TLC scanner

Exp No.	Rf			
	^{68}Ga impurities	^{68}Ga-DOTATATE	^{68}Ga-DOTATOC	^{68}Ga-DOTANOC
1	0.14	0.47	0.53	0.44
2	0.15	0.47	0.53	0.43
3	0.15	0.45	0.53	0.45
4	0.15	0.46	0.51	0.46
5	0.14	0.46	0.51	0.42
AVG	0.15	0.46	0.52	0.44
SDEV	0.01	0.01	0.01	0.02

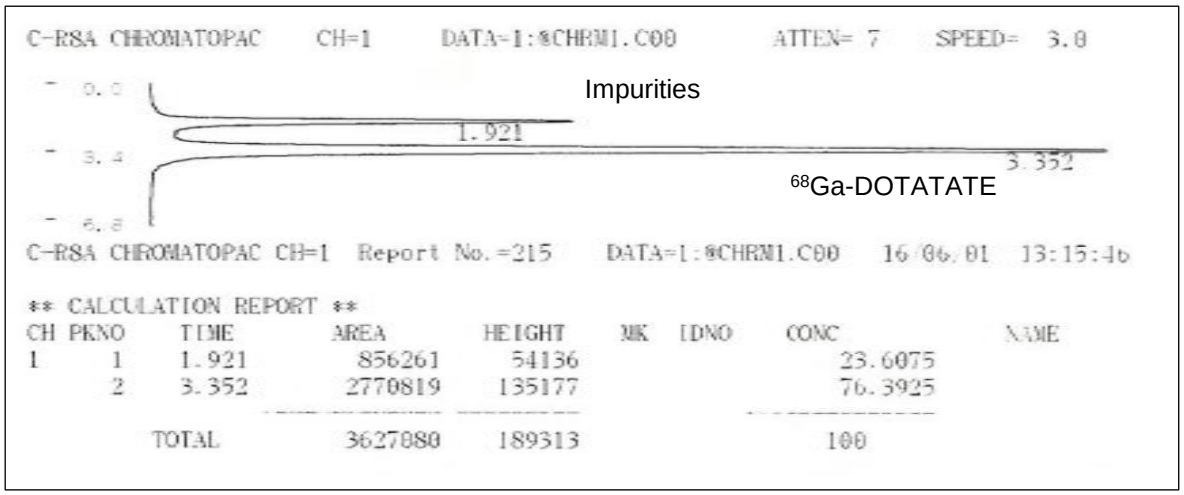


Figure 12: Typical iTLC chromatogram of ^{68}Ga-DOTATATE before Sep-Pak purification using 1 M ammonium acetate : methanol (1:1)

4.2.2.4 Testing for colloids

Labelling was performed on reaction mixtures at pH 5.0 (Table 12) and 3.5 – 4.0 (Table 13). Samples were analysed by means of HPLC and iTLC using mobile phase 1 and mobile phase 2. Each peptide was analysed once using each analytical method. An initial study with Silica gel TLC plate and mobile phase 2 did not separate the peaks very well (Figure 12) while iTLC gave two clearly separated peaks (Figure 13).

TABLE 12: The radiochemical purities of ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC after Sep-Pak purification using iTLC and HPLC

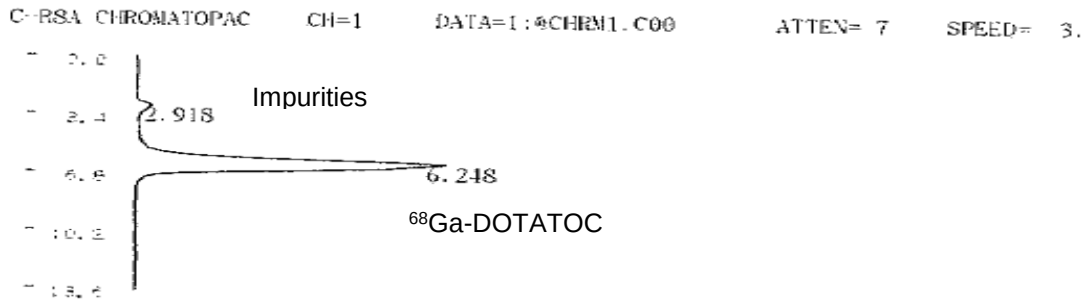
Radiolabelling was performed at pH 5.

	Mobile phase 1 % peptide	Mobile phase 1 % $GaCl_3$	Mobile phase 2 % peptide	Mobile phase 2 % impurities	HPLC % peptide	HPLC % $GaCl_3$
^{68}Ga-DOTATATE	100	0	94	6	100	0
^{68}Ga-DOTATOC	100	0	88	12	100	0
^{68}Ga-DOTANOC	100	0	81	19	100	0

TABLE 13: The radiochemical purities of ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC after Sep-Pak purification using iTLC and HPLC

Radiolabelling was performed at pH 3.5 – 4.0.

	Mobile phase 1 % peptide	Mobile phase 1 % $GaCl_3$	Mobile phase 2 % peptide	Mobile phase 2 % impurities	HPLC % peptide	HPLC % $GaCl_3$
^{68}Ga-DOTATATE	100	0	89	11	100.0	0
^{68}Ga-DOTATOC	100	0	94	6	100.0	0
^{68}Ga-DOTANOC	100	0	99	1	100.0	0



C-RSA CHROMATOPAC CH=1 Report No.=422 DATA=1:¥CHRM1.C00 16/11 26 12:1

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	2.918	16021	1042			0.9486	
	2	6.248	1672904	39113			99.0514	
		TOTAL	1688925	40155			100	

Figure 13: Typical iTLC chromatogram of ^{68}Ga-DOTATOC after Sep-Pak purification using 1 M ammonium acetate : methanol (1:1) and iTLC-SG

Chapter 5: Discussion and Conclusion

5.1 Generator Eluate

5.1.1 ^{68}Ga Yield

The change in ^{68}Ga yield with time, of the two iThemba LABS generators evaluated in this study, is depicted in Figure 3. The ^{68}Ga yield decreased from 132.0 % at the start of use to 87.8 % after 12 months (generator A) and from 125.0 % to 85.0 % (generator B). According to Meyer et al. [35] the decrease in ^{68}Ga yield could be due to changes that occur in the resin which result in ^{68}Ga being more tightly bound and therefore less is eluted. However, a pyrogallol/formaldehyde type generator and a titanium dioxide-based generator were used for this study. The ^{68}Ga yield of the Obninsk ^{68}Ge/^{68}Ga generator is 75 % after the first elution. A study by Asti et al. [15] demonstrated that this value decreased to 69 % after 7 months. The Eckert and Ziegler Galliapharm generator is greater than 95 %, the Galli Eo generator is 70 % – 75 % initially and decreases to ≥ 60 % after 12 months and the ITG generator is greater than 80 % at calibration time. The performance of the iThemba LABS generator remains within the European Pharmacopoeial limits and compares with and even exceeds that of some of the other generators on the market.

5.1.2 ^{68}Ge Breakthrough

The germanium breakthrough in generator A increased from 0.0003 % to 0.1560 % and from 0.0004 % to 0.2672 % in generator B after 12 months (Figure 4). This study shows that the breakthrough exceeds the European Pharmacopoeial limit of 0.001% by 120 days after start of use. A spike observed at 300 and 360 days in

both generators occurred after a period of non-elution of the generators due to public holidays. Increase in ^{68}Ge breakthrough was also observed and reported by users of the iThemba LABS ^{68}Ge/^{68}Ga generator after periods of non-elution [J le Roux, personal communication Oct 2016]. Sudbrock [17] used an iThemba LABS generator and also demonstrated that the ^{68}Ge breakthrough increased over time but even if it exceeded the European Pharmacopoeial limit of 0.001 % in the eluates it did not exceed it in the labelled compound because it was removed during the post purification step of the labelling process. M. Asti [15] evaluated a ^{68}Ge/^{68}Ga generator from Cyclotron Company Obninsk over a period of 7 months. The ^{68}Ge breakthrough increased by from 0.011% to 0.026%. However, after passing the eluate through a C-18 cartridge containing a cation exchange resin the ^{68}Ge breakthrough decreased by a factor of 105 in the purified eluate. Karen McElvany and co-workers [14] evaluated ^{68}Ge breakthrough and elution yields of three different types of ^{68}Ge/^{68}Ga generators (alumina/EDTA, ionic alumina and tin dioxide) for at least one year. Results showed that the elution yields of the EDTA ^{68}Ge/^{68}Ga generator dropped significantly over time while the tin dioxide generator provided consistently high yields of ^{68}Ga with low ^{68}Ge breakthrough and chemical impurities. ^{68}Ge breakthrough of the iThemba LABS generator increases with time but this does not pose a problem in the radiolabelled product if the ^{68}Ge is reduced by a purification step. The user must however be aware of the breakthrough because it poses a waste problem. According to the United States Nuclear Regulatory Commission, the release of ^{68}Ge to sewers is limited to 6.00 x 10^{-4} µCi/ml (22.2 Bq/ml) (monthly average concentration) [37]. Regulations may differ from country to country. It is clear that waste from generator elution or ^{68}Ga

labelling procedures cannot be discarded immediately. The user must have the facility to store the waste until it has decayed to acceptable levels.

5.1.3 Metal Contaminants

Eight metals were tested for (Zn, Fe, Al, Sn, Ge, Ga, Ti and Cu) and the total metal contaminants generally remained below 10 ppm (5.41µg/GBq for a 50 mCi generator). The results are shown in Table 3 and Table 4 and Figure 5. The only exception occurred in Generator B at 360 days, where the total metal content was 13.48 ppm. The high zinc concentration (10.15 ppm) contributed to the much higher total. Zinc ions will always be present in the eluate of ^{68}Ge/^{68}Ga generators because ^{68}Ga decays to ^{68}Zn. A period of non-elution would lead to more in-growth of ^{68}Zn in the generator [3], which would contribute to an increase in ^{68}Zn in the eluate. Therefore, the amount of zinc in the eluate depends on the time between elutions. Zinc content can be minimised by eluting the generator daily. Zn^{3+} and Fe^{3+} compete with Ga^{3+} during the labelling process and must therefore be minimal.

If the breakthrough and metal contaminants in the eluate exceed the required limits (> 0.001 % for ^{68}Ge breakthrough and 10 µg/GBq Fe and Zn individually), then the quality of the generator eluate can be improved by means of either fractionation, cation exchange or anion exchange chromatography [36]. In the fractionation method the activity would be concentrated in a smaller volume and therefore metal contaminants would be less. In a study performed by Asti et al. the zinc content of eluates was approximately 10^2 µg/L and iron 10^3 µg/L. After passing the eluate through a C-18 cartridge containing a cation exchange resin

there was a 95% reduction in metal contaminants including zinc and iron. The ^{68}Ge breakthrough decreased by a factor of 10^5 in the purified eluate [15].

5.1.4 Sterility and Endotoxins

Sterility: No visible bacterial or fungal growth was detected in any of the eluates after 14 days of incubation using both tryptic soy broth and thioglycolate broth. The microbiological tests in this study have therefore shown that the generator eluate remains sterile for a period of 1 year in the case of both generators. This confirms what Petrik et al. [20] stated i.e. the ^{68}Ge/^{68}Ga generator eluate is not conducive to the growth of microorganisms including *Helicobactor pylori* (which proliferates in very acidic medium) and *Deinococcus radiodurans* (which is able to proliferate in a radioactive medium).

Endotoxins: Endotoxin levels remained below 2 EU/ml. This is within the European Pharmacopoeial limit of 175 EU/V. See figure 6.

5.2 Analysis of ^{68}Ga-labelled peptides

From table 5, the average HPLC Rt of $^{68}GaCl_3$ was 2.901 minutes, the average Rt of ^{68}Ga-DOTATATE was 12.125 minutes, the average retention time of ^{68}Ga-DOTATOC was 12.178 minutes and the average Rt of ^{68}Ga-DOTANOC was 12.760 minutes. Clear separation was obtained between $^{68}GaCl_3$ and ^{68}Ga-labelled peptides. The retention times of the peptides are very close together but this does not pose a problem because only one peptide is labelled at a time. The small difference in retention times of the labelled peptides is probably due to the similarity in the molecular structure.

In a comparison of dried versus undried iTLC strips, significant differences were found in the case of ^{68}Ga-DOTATATE and ^{68}Ga-DOTANOC. However, the peaks of these peptides and $^{68}GaCl_3$ could be clearly separated. Despite the statistically significant difference, in practice either undried or dried iTLC may be used.

The iTLC retention factor (Rf) data for free ^{68}Ga, as well as for the labelled peptides, are given in Table 6 and are fairly consistent. For the sake of institutions lacking instrumentation such as a TLC scanner, data were also presented where developed plates were cut in segments and the activity of each segment was determined (see Tables 7, 8 and 9). The results were also graphically represented in Figures 9, 10 and 11. The data shows the distribution of free $^{68}GaCl_3$ and labelled peptide after chromatography and also show a clear separation between free ^{68}Ga and labelled peptide (see Figure 8). The major part of the activity, associated with the labelled peptides (average 95 - 97 %), is concentrated in the segments 1 to 7, while about 3 – 5 % of the radioactivity, corresponding to free ^{68}Ga, is concentrated in segments 8 to 9. The percentage of ^{68}Ga-labelled peptide peaks determined by radio-TLC scanner was found to be 96 – 99 %. This ‘cut and count’ method could therefore be used to determine Rf if a TLC scanner is not available. Using the strips described in our work, the strips can probably be cut at 7 cm from the bottom of the strip. The bottom section would represent the labelled peptide and the top section the free $^{68}GaCl_3$.

Table 10 shows results for iTLC analysis performed using each of the three radio-labelled peptides spiked with 2 % $^{68}GaCl_3$. Clear separation of $^{68}GaCl_3$ from labelled peptide using different amounts will demonstrate the sensitivity of the

method. If similar results are obtained using HPLC, then the iTLC method which is quicker than the HPLC method can be used in instances where there is a time constraint or if no HPLC is available. The percentage of $^{68}GaCl_3$ obtained using HPLC was always higher than 2 % while the percentage of $^{68}GaCl_3$ obtained for the same sample using iTLC was always found to be less than 2 %. It is safer to use the HPLC method which overestimates the actual result than a method that underestimates the impurity.

The results in Table 11 show the Rf values obtained for $^{68}GaCl_3$, ^{68}Ga-DOTATATE, ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC using mobile phase 2. Although sharp peaks were achieved with this method, a slight overlap of peaks occurred between $^{68}GaCl_3$ and ^{68}Ga-peptides. An example of a chromatogram is displayed in Figure 12. This method was performed on a TLC plate and was developed for 30 minutes as opposed to the iTLC method (mobile phase 1) in which the strip was developed for 3.5 minutes. When iTLC was performed with mobile phase 2 on iTLC paper the development time was approximately 10 minutes and clear separation was obtained between peaks. It is therefore recommended that iTLC paper be used with mobile phase 2 instead of silica gel plates. According to Mukherjee [32], the ammonium acetate/methanol mixture on paper was able to separate colloidal gallium from labelled peptides. Our study with peptides labelled at higher pH to induce colloid formation (Tables 12 and 13) confirmed that iTLC-SG with mobile phase 2 is also suitable to distinguish between colloidal ^{68}Ga and ^{68}Ga-labelled peptides. In contrast, iTLC using mobile phase 1 and HPLC showed a higher radiochemical purity because colloids cannot be separated from the ^{68}Ga-labelled peptides with these methods. It is interesting

to note that the Sep-Pak C-18 cartridge did not remove all impurities formed at a higher pH. We have no explanation for the high level of impurities formed in ^{68}Ga-DOTATATE at pH 3.5 – 4.0.

Weaknesses of this study

- Ideally, more generators should have been evaluated. However, only one generator was available per year because customer requirements were also considered.
- Further experiments should be performed to determine the lower limit of ^{68}Ga that can be detected using the iTLC method.
- No clear separation was obtained between $^{68}GaCl_3$ and ^{68}Ga-labelled peptides when using mobile phase 2 and TLC plates. This aspect should be investigated further. More experiments with mobile phase 2 and iTLC-SG strips should also be done to confirm the findings of the limited work done in this study.

Strengths of this study

- Other studies have been performed to evaluate the iThemba LABS generator but none of them was as comprehensive as the current work. In the study performed by McElvany et al. [14] the ^{68}Ge breakthrough and metal contaminants were compared over a period of 1 year but the generator was eluted with 1 M HCl instead of 0.6 M HCl as recommended by the manufacturer. Das et al. [16] performed an evaluation of a tin dioxide generator over a period of six months. Random eluates were used to monitor elution efficiency, ^{68}Ge breakthrough and ^{65}Zn content. Sudbrock et

al. [17] used four iThemba LABS generators to evaluate the ^{68}Ge breakthrough over time. Three of the generators were evaluated over a period of nine months while the fourth generator was evaluated over a period of eight months. ^{68}Ge breakthrough was determined on 123 eluates and 115 ^{68}Ga-DOTATATE samples. In our study a comprehensive evaluation which included ^{68}Ga yield, ^{68}Ge breakthrough, metal contaminants, endotoxins, sterility and samples were analysed on a monthly basis over a one year period.

Recommendations for the user

Generator

There are differences between generators from different manufacturers and this will affect aspects of use including the eluents, impurities, purification methods, labelling methods, safety and waste disposal.

The generator should be eluted daily to avoid increased ^{68}Ge and zinc ions in the eluate. If the generator has not been eluted daily, then it should be eluted at least once before the eluate is used for radio-labelling.

Some form of purification of the eluate or fractional elution is recommended for the iThemba Labs generator.

Users should also be aware of the ^{68}Ge breakthrough especially with regards to waste disposal.

Chromatography

Either dried or undried iTLC strips can be used.

In cases where a radio-TLC scanner is not available, strips can be cut in two segments and the activity on each strip can be determined using a dose calibrator. For the 9 cm strips in this work, cutting at 7 cm from the bottom of the strip would be appropriate.

Conclusion

This study demonstrates that the iThemba LABS $^{68}Ge/^{68}Ga$ generator remains stable over a period of at least one year with the yield, metal contaminants, sterility and endotoxin concentration still within the European Pharmacopoeial limits. The breakthrough increases to above the specification but can be reduced by fractionated elution or by using anion and/or cation exchange chromatography. However, to obtain consistent, reliable results the generator must be eluted daily or at least a few times before labelling if there has been a period of non-elution. Even though the very acidic environment of the generator is not conducive to the growth of microorganisms, the generator should be stored and eluted in a biohazard cabinet in a clean room to ensure the sterility of the eluate. The eluate can be used for a period of up to one year but by that time the amount of ^{68}Ga eluted will be very low (less than 185 MBq for a 1850 MBq generator). Using it will therefore depend on the amount of ^{68}Ga activity required.

Clear separation was obtained for $^{68}GaCl_3$ and ^{68}Ga-lablled peptides using both iTLC with citrate buffer and HPLC, but the HPLC method proved to be more reliable for the determination of $^{68}GaCl_3$ in ^{68}Ga-DOTA peptides. It was demonstrated that the iTLC strips could be cut into segments and the segments measured on a dose calibrator for situations where there is no HPLC or TLC scanner available. The results also show that it is recommended but not essential to use dried chromatography paper for iTLC. The iTLC method using ammonium acetate/methanol seems to be the optimal system for routine analysis of ^{68}Ga labelled DOTA-peptides, as it separates both $^{68}GaCl_3$ and colloidal impurities from the labelled peptides and is a fast and easy technique.

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