A comparison of labelling characteristics of manual and automated synthesis methods for gallium-68 labelled ubiquicidin

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Highlights

- Optimized manual and automated synthesis methods for [^{68}Ga]Ga-NOTA-UBI were compared.
- Automated methods were more robust than a manual method.
- Operator radiation exposure was considerably less for automated synthesis methods.

Abstract

Gallium-68 labelled 1,4,7-triazacyclononane-1,4,7-triacetic acid ubiquicidin (NOTA-UBI) is currently investigated as a PET radiopharmaceutical for the imaging of infections. The aim of this study was to compare the labelling characteristics of an optimized manual radiosynthesis method with those of optimized automated synthesis methods. Data from this study suggest that automated radiosynthesis of [^{68}Ga]Ga-NOTA-UBI provides a higher degree of robustness and repeatability than the manual method. Our results also suggest that for our full-scale automated synthesis, radical scavengers should be considered to reduce radiolysis. Automated synthesis methods have the advantage of markedly reducing radiation exposure to operators. Standardised automation also makes the synthesis more reliably compliant with Good Manufacturing Practice guidelines.

Keywords: Ga-68; Automated synthesis; Manual synthesis; NOTA-UBI; Tin-dioxide generator; Radiation exposure

1. Introduction

The development of synthesis methods for novel radiopharmaceuticals often entails testing of different manual radiolabelling procedures on a small scale. Small-scale labelling has the advantage that it limits the radiation exposure to the operator (De Decker and Turner, 2011). Initial experiments are usually repeated several times to assess the impact of various changes such as pH, incubation temperature and time, type and volume of buffer etc. on the success of the radiosynthesis. Optimizing radiometal-based synthesis methods may also include the evaluation of bifunctional chelators to determine the best radiometal-chelator-ligand complex. Once optimal labelling conditions have been determined and satisfactory results achieved, the next phase typically comprises evaluation of the robustness and repeatability of the radiosynthesis evaluation. This phase often includes up-scaling the quantity of reagents together with an increase in radioactivity used. Scaling-up is required to determine if labelling results can be reproduced using sufficient radionuclide for one or more patients. In theory, labelling results obtained from manual labelling methods should correlate well with those obtained from automated syntheses.

Ubiquicidin 1-59 is a 6.6 kD linear cationic peptide with antimicrobial properties. It has been shown to be present in low concentration in various organs such as the colon mucosa, epithelial cells of the human airways and also macrophages (Hiemstra et al., 1999; Tollin et al., 2003). It is present intra-cellularly and only released during acute infection or severe cell damage. Ubiquicidin (UBI) has been found to affect a spectrum of pathogens (Brouwer et al., 2006). Various peptide fragments of UBI 1–59 have been synthesized, including UBI 1–18, UBI 18–35, UBI 18–29, UBI 29–41 and UBI 31–38. Brouwer's study also showed that the synthetic UBI derivatives containing amino acids 29–41 or 31–38 had the best targeting properties which could possibly be adopted as a tool for non-invasive nuclear imaging techniques such as single-photon emission tomography (SPECT) and positron emission tomography (PET). Researchers have since then developed a number of procedures that can be used for radiolabelling of ubiquicidin fragments (Bhatt et al, 2017, 2018; Bhusari et al., 2019; Brouwer et al., 2006; Vilche et al., 2016). Peptide fragments UBI 29–41 and UBI 31–38 were successfully labelled with gallium-68 and technetium-99m (^{99m}Tc). Preclinical investigations in mice by Brouwer et al. have shown that the ^{99m}Tc-labelled fragments accumulated only in sites of infection.

There are a number of approaches that can be used in the production of gallium-68 (^{68}Ga) radiopharmaceuticals for clinical application (Velikyan, 2015). Automated and semi-automated radiosyntheses of ^{68}Ga-radiopharmaceuticals have the advantage of enabling compliance with Good Manufacturing Practice (GMP) guidelines (Vis et al., 2015).

In 2014, synthesis of gallium-68-labelled UBI 29–41 (i.e. [^{68}Ga]Ga-NOTA-UBI) using a manual radiolabelling technique was first reported (Ebenhan et al., 2014) and a module-based automated radiosynthesis has recently been developed (Le Roux et al., 2020). The use of automated synthesis modules to label radiopharmaceuticals with ^{68}Ga has been reported by several authors (Decristoforo, 2012; Malizia et al., 2012; Schopf et al., 2018; Vis et al., 2015). Well-known buffers such as sodium acetate, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) and sodium formate are commonly used in ^{68}Ga-labelling (Bauwens et al., 2010; Sasson et al., 2010; Velikyan et al., 2008).

Various methods described in the literature compare manual with automated syntheses but to the best of our knowledge no head-to-head comparison of a manual synthesis with two automated methods for the same radiopharmaceutical exists. The radiochemistry of the two automated labelling methods differ in terms of ^{68}Ga-eluate processing and type of buffer used. The effect of these differences on the labelling characteristics and choice of a radical scavenger are also highlighted. The aim of this study was to evaluate how the labelling characteristics of a manual synthesis procedure for preparing [^{68}Ga]Ga-NOTA-UBI compare to those of two different automated synthesis procedures.

2. Methods

2.1. Material and preparations

Analytical or pharmaceutical grade reagents were used for both manual and automated synthesis procedures. Gallium-68 eluates were obtained from ^{68}Ge/^{68}Ga-generators with nominal activity 1850 MBq (iThemba LABS, Somerset West, South Africa) using 0.6 M HCl (ABX, Radeberg, Germany). Both the generator and the GRP automated synthesis unit (Scintomics, Fürstenfeldbruck, Germany), were housed in a NMC Ga-68 hot cell (Tema Sinergie, Faenza, Italy). All disposable material for the synthesis module was compliant with GMP standards. The routine preparation and general radiosynthesis protocols using a module for the production of [^{68}Ga]Ga-NOTA-UBI were previously reported (Le Roux et al., 2020).

Freeze-dried batches of UBI 29–41, conjugated to either 1,4,7,10-tetra-azacyclododecane-1,4,7,10-tetra-acetic acid (DOTA) or 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) or 1,4,7-triazacyclononane, 1-glutaric acid-4,7-acetic acid (NODAGA), were obtained from GL Biochem (Shanghai, China) and stored at −80 °C as 25 µg aliquots suitable for small-scale, manual radiolabelling. For the automated methods, 2 mg of NOTA-UBI (ABX, Radeberg, Germany) was dissolved in 2.0 ml of ultra-pure water to render a stock solution with a concentration of 1 mg/ml. The stock solution was sub-divided in 50 and 100 µl aliquots and frozen at −20 °C. A sodium acetate trihydrate (NaOAc) solution (2.5 M) was used as a buffer for manual preparations while 1.0 M NaOAc or 1.0 M ammonium formate were used in the automated synthesis methods. Based on initial experiments, aliquots of NOTA-UBI were prepared for up-scaled production at 50 µg (all automated syntheses using generator eluate fractionation) and 100 µg (all automated syntheses including eluate pre-processing), to meet the criteria for optimal [^{68}Ga]Ga-NOTA-UBI preparation under the different radiolabelling conditions. Ascorbic acid and pharmaceutical grade ethanol were obtained from North East Pharmaceutical Group (Midrand, South Africa) and Merck (Kenilworth, NJ, USA) respectively. PS-H$^+$ cartridges (ABX, Radeberg, Germany) were used for eluate pre-processing. C18 SEP-Pak cartridges (Waters, Milford, MA, USA) were used for purification post-radiolabelling if required.

2.2. Radiolabelling

Manual radiolabelling: Small-scale and up-scaled manual radiosyntheses were used as references, adopting the conditions described previously (Ebenhan et al, 2014, 2018) using ^{68}Ge/^{68}Ga-generator eluate fractionation. The first set of experiments investigated the ^{68} Ga-

radiolabelling of DOTA-UBI, NODAGA-UBI and NOTA-UBI to find the most suitable chelator-ligand complex. Concentrations (µg/ml) of 4, 8 16, 32, and 64 for each chelator-ligand complex were labelled with micro-scale quantities of gallium-68. Briefly, 180 µl of buffered ^{68}Ga solution (pH 3.5–4.0) was added to either of the NOTA-, NODAGA- and DOTA-UBI solution. The labelling mixtures were heated for 10 min at 80 °C, followed by a cooling period of 5 min. The scaled-up radiolabelling conditions remained the same; however, 2 ml ^{68}Ga activity (587 ± 149 MBq) was mixed with 25 µg NOTA-UBI. The NOTA-UBI concentration of the reference manual labelling was 19.6 µg/ml.

Automated radiolabelling: Method 1 used fractional generator elution while an eluate pre-purification step with a strong cationic ion exchanger was used for automated method 2. Importantly, both manual and automated methods were optimized for type of buffer used, peptide mass and use of radical scavengers, if required.

Automated method 1: ^{68}Ge/^{68}Ga-generator eluate fractionation (1498 ± 73 MBq, with an approximate loss of 12% due to fractionation) was performed with 0.6 M HCl and a 2 ml fraction was used for the synthesis. The pH of the eluate was adjusted to 3.5–4.0 using 2.0 ml of a 1.0 M ammonium formate buffer. The buffered eluate mixture was slowly added to the reaction vial containing 50 µg of NOTA-UBI and 350 µl 1.4% ascorbic acid. The NOTA-UBI concentration in this labelling mixture was 11.2 µg/ml. The mixture was heated for 10 min at 90 °C, cooled for one minute and purified using a C18 Sep-Pak cartridge. [^{68}Ga]Ga-NOTA-UBI was desorbed from the C18 matrix using a mixture of ethanol/saline (50% v/v) and ultimately filtered through a 0.22 µm filter into a sterile vial.

Automated method 2: The ^{68}Ge/^{68}Ga-generator was eluted with 10 ml 0.6 M HCl. The eluate (1467 ± 85 MBq) was further diluted to 18 ml with ultra-purified water and slowly passed over a PS-H+ cartridge where most of the ^{68}Ga activity was trapped and purified from co-eluted metals. Subsequently, 1.5 ml of a 5.0 M NaCl solution was used to desorb the ^{68}Ga-activity from the PS-H+ matrix into a reaction vessel containing 100 µg NOTA-UBI buffered in 1.3 ml 1.0 M NaOAc. The reaction vessel also contained a scavenger combination of 350 µl 1.4% ascorbic acid and 170 µl ethanol. This radiolabelling mixture, with a NOTA-UBI concentration of 28.4 µg/ml, was further processed as described above in method 1.

2.3. Qualitative analysis

Radio-high performance liquid chromatography (radio-HPLC).

Following completion of the automated methods the radiochemical purity (RCP) was determined using radio-HPLC with a Shimadzu Nexera XR HPLC system (Kyoto, Japan), which included a variable wavelength photodiode array detector and a gamma detector (Raytest, Straubenhardt, Germany). The solvent gradient method consisted of mobile phases (v/v) A (0.1% trifluoroacetic acid (TFA) in water) and B (0.1% TFA in acetonitrile) using a Waters C18 analytical column (4.6 mm × 250 mm x 5 µm) as stationary phase (0–2 min: 5% B, 2–18 min: 65% B, 18–24 min: 5% B). The flow rate was set at 2.0 ml/min. The column temperature was kept at 40 °C throughout the analysis. The retention time for [^{68}Ga]Ga-NOTA-UBI was 6.0–6.9 minutes whereas free ^{68}Ga-activity was retained for 1–2 minutes.

Radio-instant thin-layer chromatography (radio-ITLC).

A glass microfiber chromatography strip impregnated with silica gel (ITLC-SG, Agilent Technologies, Folsom, CA, USA) was used as stationary material. The mobile phase consisted of 0.1 M sodium citrate (pH = 5.0). Distribution of radioactivity was determined using a radio-chromatographic scanner (Lablogic, Sheffield, United Kingdom) allowing gamma-counting of the full length of the ITLC-SG strip. Radiochemical purity was determined by peak analysis (counts-per-minute/area under the curve). [^{68}Ga]Ga-NOTA-UBI remained at the origin whereas unchelated ^{68}Ga was separated by migrating with the mobile phase front.

2.4. Product stability

Stability at room temperature of [^{68}Ga]Ga-NOTA-UBI prepared by both automated methods was assessed following sterile filtration and dilution to 15 ml with phosphate buffered saline. The radiochemical purity was determined with radio-ITLC and radio-HPLC analyses at the end of synthesis (EOS) and at 180 min.

2.5. Additional quality control measures

Table 1 presents a summary of additional quality control procedures performed after module-based synthesis to ascertain if the final product complied with our in-house release criteria.

Table 1. Quality control procedures and release criteria for automated radiosynthesis methods.

Quality control procedure	Release criteria	Method
Visual appearance	Clear, colourless, particle free	Visual inspection
Radiochemical purity	≥95% [^{68}Ga]Ga-NOTA-UBI	ITLC /HPLC
Radionuclidic identity (half-life)	63–73 min	Dose calibrator
pH of final product	6.0–8.0	pH strips
Bacterial endotoxins	<10 EU/ml	Endosafe PTS[a]
Residual ethanol content	<10% v/v	Calculation
Sterile product filtration	≥3.45 bar	Filter integrity test
Bacterial growth	Sterile (pass)	Broth incubation
Germanium-68 breakthrough[b]	<0.001%	Dose calibrator

[a]Charles River Laboratories, Wilmington, Massachusetts, USA.
[b]^{68}Ge breakthrough was measured 48 hours post synthesis using the residual activity that remained in the vial after sampling for quality control.

2.6. Operator radiation exposure

All radiolabelling techniques were performed using appropriate radiation protection and shielding. The guidelines of the European Pharmacopoeia (Ph. Eur., 2017) were followed for preparation of the ^{68}Ga-radiopharmaceuticals. The fully-shielded, automated synthesis module was remotely operated. The ^{68}Ge/^{68}Ga-generators used for this part of the study

had a similar age of three months. Operator radiation exposure was recorded using an electronic X-ray and gamma personal dosimeter (PM1610, Polimaster, Belarus) (energy range of 29 keV–10 MeV; 0.1 μSv increments) attached to the outside of the lab coat. The whole-body radiation dose was determined by recording the dosimeter reading at the beginning of the elution until end of synthesis, including mimicking the dispensing of two patient doses in a shielded laminar flow unit.

3. Results and discussion

Prior to this comparative study, each of the automated synthesis methods was optimized for peptide mass, buffer type and volume as well as scavenger addition. This resulted in different NOTA-UBI concentrations for the 3 labelling methods. Experimental results during the development of the automated synthesis methods showed the formation of a radiolytic impurity (retention time ~ 6.1 min) seen on HPLC (data not shown) which required the use of an appropriate radical scavenger. The peptide sequence of ubiquicidine (TGRAKRRMQYNRR) contains a methionine group which is known to be prone to radiolysis (Mueller et al., 2016). The manual radiolabelling did not necessitate the use of a scavenger.

3.1. Choice of chelator-UBI complex

The optimal combination of azamacrocyclic chelators (NOTA-NODAGA and DOTA) and UBI was identified by gradually decreasing the chelator-UBI concentration during manual radiolabelling to 4 μg/ml (Fig. 1). The best RCP obtained using the NOTA moiety was compared to that of NODAGA- and DOTA-UBI ($p < 0.05$). To determine the optimal labelling concentration that yielded a RCP >90%, chelator-UBI concentrations (μg/ml) of ≥8, ≥16 and ≥ 32 for NOTA-UBI, NODAGA-UBI and DOTA-UBI were evaluated. Such a direct comparison has not yet been described in the literature; however, experimental work conducted by Guérin et al. also suggested the use of NOTA-functionalized peptides for PET imaging (Guérin et al., 2010). Tri-azamacrocyclic bifunctional chelators such as NOTA and NODAGA have both been suggested as viable alternatives to DOTA based on the formation of thermodynamically stable complex with Ga(III) ions. Maximal interaction is achieved due to optimal N_3O_3 denticity and a smaller ring size than that of DOTA. Subsequently, the successful synthesis of NODAGA-UBI with ^{68}Ga was also reported (Bhatt et al., 2017).

A total mass of 25 μg NOTA-UBI was deemed sufficient for an up-scaled manual labelling to account for precursor losses due to glassware adherence and possible slower reactivity in the larger reaction volume. Table 2 summarises the results obtained with the manual synthesis method versus the two automated synthesis methods. The ^{68}Ga activity for the manual radiolabelling was 458–806 MBq, considered to be an acceptable range of radioactivity for radiation protection and efficient dose preparation. For automated methods, the average ^{68}Ga starting activities were 1483 ± 81 MBq. In automated method 1, due to fractionation, the actual activity added to the peptide was slightly lower.

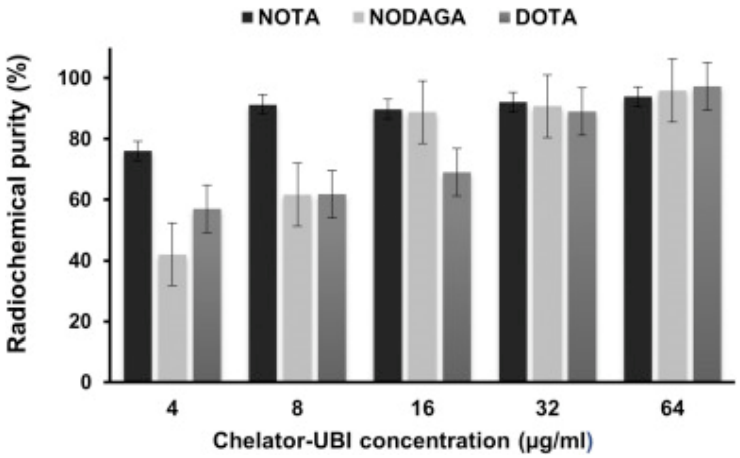

Fig. 1. ^{68}Ga radiosyntheses of UBI conjugated to NOTA, NODAGA or DOTA as azamacrocyclic bifunctional chelators (n = 3); radiolabelling was performed in the presence of NaOAc, pH 3.5–4, at 80 °C for 10 min. The radiochemical purity was determined using ITLC.

Table 2. Comparison of the manual method with the automated synthesis methods.

Empty Cell	**Manual radiolabelling (n = 9)**	**Automated method 1 (n = 3)**	**Automated method 2 (n = 3)**
Volume of ^{68}Ga-activity (ml)	1.0	2.0	10.0
Type of eluate pre-processing	EF	EF	SCX
Average starting activity (MBq)	587 ± 149	1498 ± 73$^{\$}$	1467 ± 85
Residual radioactivity: PS-H $^{+}$ (%)$^{\#}$	–	–	8.8 ± 2.0
Buffering molarity and type	2.5 M NaOAc	1.0 M NH_4HCO_2	1.0 M NaOAc
Volume of buffer used (ml)	0.278	2.0	1.5
Total mass NOTA-UBI (µg)	25	50	100
Incubation time (min)/(°C)	10–15/80	10/90	10/90
Radiosynthesis time (min)†	31 ± 7	32 ± 2	38 ± 2
% Radiochemical yield $^{\#}$	65.5 ± 22.6	63.2 ± 1.5	57.3 ± 3.8
% radiochemical purity (ITLC)	97.1 ± 1.9	98.9 ± 0.3	99.3 ± 0.1
% radiochemical purity (HPLC)	97.5 ± 0.8	96.4 ± 0.9	97.3 ± 0.5
[^{68}Ga]Ga-NOTA-UBI yield (MBq)	473 ± 234	690 ± 22	580 ± 99
Molar activity (MBq/nmol)	20.4 ± 11.4	27.6 ± 0.9	11.4 ± 1.9
Retained activity C18 SPE (%)	10.0 ± 8.9	1.6 ± 1.4	2.5 ± 1.5

Footnotes: EOS = End of synthesis, NH4HCO2 = ammonium formate; NaOAc = sodium acetate tri-hydrate; EF = eluate fractionation; SCX = eluate processing using a strong cationic exchange matrix; † = time measured from start of elution of generator; # = decay-corrected data; $ = total activity of eluate prior to fractionation.

3.2. Radiosynthesis time

The manual method had the shortest radiosynthesis time (31 ± 7 min) while the radiosynthesis time for the cationic pre-purification method was the longest (38 ± 2 min). Both automated synthesis methods included a 6-minute step to condition the C18 cartridge prior to generator elution (not reflected in the synthesis time). The added pre-purification step, which utilizes all eluted ^{68}Ga activity, increased the synthesis time by 6 minutes. Pre-purification usually also increases the molar activity by decreasing the volume of the labelling mixture. The loss of activity on the PS-H$^+$ cartridge was less than 10% of the eluted activity from the generator. Despite this longer synthesis time, it is deemed necessary to include this step in the synthesis methods where a ^{68}Ge/^{68}Ga-generator eluate with known high levels of metal impurities is used (Chakravarty et al., 2013). It is well-known that certain metal impurities such as Zn, Fe and Cu may have a detrimental effect on the synthesis of peptides with ^{68}Ga-chloride (Velikyan, 2015).

The increase in radiosynthesis time in method 2 resulted in about 10% decrease in radiochemical yield when compared to the automated fractional elution method.

3.3. Choice of buffering agent

Manual synthesis may be susceptible to operator influences such as the speed at which the solvents and reagents are introduced during the various synthesis steps or between different syntheses. In the automated synthesis methods, reagents and solvents are introduced at pre-programmed speeds in a consistent manner. The volume of the eluate and buffer used in the manual method was smaller than that used in the automated methods. The buffer volume was intentionally increased and the molarity of NaOAc adapted for automation because the synthesis module could not accurately add a volume less than 500 µl into the reaction vial. This increase in buffer volume resulted in varying peptide concentrations for the three synthesis methods. Variable peptide concentrations used in the automated methods still resulted in acceptable radiochemical purity and yield.

3.4. Radiochemical purity

Experimental results during the development of the automated synthesis methods showed the formation of a hydrolysis impurity seen on HPLC (data not shown). The average starting ^{68}Ga-activity for the manual method was 587 ± 149 MBq while for automated methods it was about 2–3 times higher (1483 ± 81 MBq). The higher ^{68}Ga-activities used in the automated methods made them more prone to radiolysis. In order to reduce radiolysis it was necessary to use radical scavengers in the automated methods. The addition of radical scavengers successfully reduced the radiolytic impurities and increased RCP to ≥95%. The manual method did not require the use of a scavenger. Besides lower starting activities, differences in peptide mass and volume of labelling mixture may have contributed to prevention of radiolysis. Herein, original data is provided, suggesting that the radiochemical purity of both automated methods was more robust and repeatable than that observed with the manual method. The average radiochemical purity (TLC and HPLC) for both the manual and automated procedures was above the required 95%.

3.5. Radiochemical yield and losses of product activity

The manual method resulted in varying radiochemical yields (42–89%), amongst others due to inconsistency in following the labelling protocol. However, this is not uncommon for manual radiosynthesis. The average radiochemical yield of module-based preparations of [^{68}Ga]Ga-NOTA-UBI showed a markedly smaller variation (<4%) than the manual method (22%).

There was a large difference in the percentage activity retained on the C18 cartridge when the manual method is compared to automated methods. The manual method retained 4–5 times more activity than the automated methods, despite matching labelling conditions. A possible reason can be that more colloids are formed during the manual method, which are retained on the C18 cartridge during product purification. The smaller peptide quantity (25 µg) used for the manual labelling method may bind gallium-68 less effectively, thus allowing more colloid to be formed during synthesis (Brom et al., 2016). The TLC method generally used for the detection of gallium-colloids in gallium-68 radiopharmaceuticals (stationary phase: iTLC-SG strip, mobile phase: 1 M ammonium acetate/methanol 1:1 v/v) could not distinguish between colloidal impurity and the labelled ubiquicidin. Using this method, gallium-68 colloidal impurities and labelled product remained at the origin. Various other methods consisting of iTLG-SG strips in combination with several alternative mobile phases were also investigated but a reliable method could not be identified. It should be noted that the average RCY from all methods was satisfactory, providing sufficient radioactivity to potentially prepare one or more patient doses despite differences in generator age.

3.6. Effect on final product quality and stability

The molar activity of the different radiosynthesis methods ranged from 11.4 MBq/nmol to 27.6 MBq/nmol. This wide range of molar activities did not affect the quality of the final product. Radio-ITLC and radio-HPLC results indicated that [^{68}Ga]Ga-NOTA-UBI, labelled with both automated methods, remained stable at room temperature for at least 180 min (data not shown). Unpublished data also confirmed that the manually labelled [^{68}Ga]Ga-NOTA-UBI was stable for up to 180 min post synthesis.

3.7. Radiation exposure to operator

Radiation exposure to operators is influenced by several factors such as exposure time, shielding and distance from the radiation source, as well as the degree of automation of the radiosynthesis. Module-based syntheses have a clear advantage over manual methods in this regard. Table 3 provides a summary of radiation exposure readings recorded during manual and module-based methods. In general, exposure levels for the manual method were higher despite using a lower starting activity (about 60% less). In order to compare the exposure from manual and automated syntheses, we normalized the effective doses to starting activity. The normalized effective dose for the automated syntheses was 0.002 µSv/MBq compared to 0.04 µSv/MBq for the manual method. This is a 20-fold reduction in radiation exposure. These data confirm the added advantage of automation regarding

radiation exposure of operators in the day-to-day production of radiopharmaceuticals in a clinical setting.

Table 3. Comparison of whole-body radiation exposure: manual versus automated methods.

Method (n = 5)	^{68}Ge/^{68}Ga generator shelf-life (days)	Synthesis time (min)	Effective dose (μSv)	Normalized effective dose (μSv/MBq)
Manual	109 ± 7.9	32.2 ± 7.5	25.2 ± 6.2	0.04 ± 0.05
Automated[a]	101 ± 51	36.9	2.3 ± 0.4	0.002 ± 0.01

[a]Data from method 1 and 2 were combined.

Automated synthesis modules are usually housed in hot cells which also contribute to lower operator radiation exposure and lower background radiation. Even without a hot cell, the hands-off set-up is the most important reason for the reduced radiation exposure seen with the automated methods, as there is no need for the operator to remain in the vicinity of the high radioactivity. It can be argued that a major advantage of manual syntheses is the possibility to intervene in the labelling procedure if necessary. However, operator intervention makes it more difficult to comply with GMP standards.

In a scenario where an operator uses the manual labelling method daily, the whole-body effective dose could exceed 6 mSv per year. Higher starting activities or manual labelling several times per day could lead to a whole-body effective dose close to the annual limit of 20 mSv per year (averaged over 5 years) as published by the International Commission for Radiological Protection's guidance for occupational exposure (International Commission on Radiological Protection, 2003).

3.8. Additional quality control and compliance with release criteria

Pharmacopoeias provide the legal and scientific benchmark for delivering high quality medicines, including radiopharmaceuticals. Novel radiopharmaceuticals, such as ^{68}Ga-labelled ubiquicidin, do not have pharmacopoeial monographs. This study used the European Pharmacopoeia's published monograph for Gallium (^{68}Ga) Edotreotide Injection as a guide for the release criteria for ^{68}Ga-labelled ubiquicidin, as the synthesis of these two radiopharmaceuticals is very similar (European Pharmacopeia, 2017). Both automated methods fulfilled our in-house criteria for release of a radiopharmaceutical for human administration (Table 1). Manual radiolabelling met the release criteria in three consecutive preparations. Even though it may be generally compliant with GMP, a weakness of the manual method is that it is less robust. GMP plays an integral part in the production of radiopharmaceuticals intended for clinical use. Consistent and reproducible results produced by automated synthesis methods make them the method of choice for producing radiopharmaceuticals in a GMP-compliant manner. Batch records produced by the module software are usually GMP-compliant, providing a method to monitor labelling steps and conditions. The module software furthermore provides a mechanism for reliable traceability of the radiopharmaceutical manufacturing process.

4. Conclusion

The aim of this work was to compare the product characteristics and pharmaceutical quality of optimized automated and manual methods.

Our results showed a high degree of robustness and repeatability using a Scintomics GRP synthesis unit. It was necessary to include scavengers for both automated methods to reduce radiolysis. Automated synthesis methods furthermore have the clear advantage of reducing radiation exposure to operators and facilitating production of radiopharmaceuticals in a GMP compliant manner.

Credit authorship contribution statement

Jannie le Roux: Conceptualization, Investigation, Writing - original draft, formal writing – original and final draft. Sietske Rubow: Supervision, Writing - review & editing. Thomas Ebenhan: Writing - review & editing

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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