

Synthesis, validation and quality controls of [⁶⁸Ga]-DOTA-Pentixafor for PET imaging of chemokine receptor CXCR4 expression

Antonino Sammartano[§], Silvia Migliari, Maura Scarlattei, Giorgio Baldari, Livia Ruffini

Nuclear Medicine and Molecular Imaging Department, Azienda Ospedaliero-Universitaria di Parma, Italy

[§]For correspondence: ansammartano@ao.pr.it, phone number: 0039 3317867654

Summary. Chemokine receptor-4 (CXCR4) is involved in tumor growth and progression in several types of human cancer. Recently, [⁶⁸Ga]-DOTA-Pentixafor has been assessed as an excellent imaging probe targeting CXCR4-expression using positron emission tomography (PET). Here we report on the entire production cycle of [⁶⁸Ga]-DOTA-Pentixafor, including quality control development and process validation. *Methods.* Synthesis of [⁶⁸Ga]-DOTA-Pentixafor was validated *via* three independent and consecutive production runs using an automated synthesis system. All validation runs must pass the pre-set quality control (QC) limits. Validation was performed for established QC tests to ensure that methods were reproducible and reliable in routine use. Germanium-68 breakthrough was determined for each sample. Production yield was calculated for each synthesis to assess the performance and efficiency of the radiolabeling process. The quality of the final product was determined by ITLC and HPLC methods after each synthesis. *Results:* The average ITLC-measured radiochemical purity was above 98.5% and HPLC-measured radiochemical purity was 99.86%, 99.83% and 100% in the three validation runs. Germanium breakthrough was 4.8*10⁻⁵%, 4.9*10⁻⁵% and 4.7*10⁻⁵% of total activity, far below the recommended level of 0.001%. Residual ethanol resulted 5.22%, 5.58% and 5.32%V/V; spot of HEPES impurity was not more intense than spot of reference solution (200µg/V). Endotoxin level resulted <17.5EU/ml. pH of the final product was 7 in all samples. *Conclusion:* [⁶⁸Ga]-DOTA-Pentixafor fit requirements of the pre-set quality parameters of purity, efficacy and safety in the batches considered for this study and fulfilled all the acceptance criteria for injectable radiopharmaceutical products. The results demonstrated a batch-to-batch reproducibility providing high radiochemical purity. (www.actabiomedica.it)

Key words: CXCR4 targeting, PET, radiopharmaceutical validation, [⁶⁸Ga]-DOTA-Pentixafor.

Introduction

The C-X-C chemokine receptor 4 (CXCR4) and its ligand CXCL12 are overexpressed in a variety of tumor types, strongly promoting tumor growth, invasiveness and metastasis through multiple signal pathways [1].

Moreover, the CXCR4/CXCL12 axis is a key factor involved in the process of cell migration at sites of infection and/or inflammation [2-3].

Recently, new imaging probes targeting CXCR4 have been developed for PET imaging of several different hematologic and other neoplasms including leukemia, lymphoma, multiple myeloma, adrenocortical carcinoma or small cell lung cancer and also in other solid tumors and disease conditions, such as splenosis, stroke, atherosclerosis, and myocardial infarction in humans and in animals [4-6].

Among CXCR4-directed imaging agents, Pentixafor labeled with Gallium-68 (Ga-68) has shown

a unique position, because of its elevated affinity and selectivity to CXCR4, its low unspecific binding and adequate distribution profile accompanied by quick renal excretion [7-8].

Combined with a favorable dosimetry, these characteristics paved the way for first currently ongoing clinical studies for high contrast PET imaging of CXCR4 expression.

Starting on April 2019 our Radiopharmacy Laboratory have set up the synthesis method and quality control (QC) procedures of the PET probe [^{68}Ga]-DOTA-Pentixafor for molecular imaging of the CXCR4/CXCL12 axis.

In recent years, various new diagnostic agents based on Ga-68 labeling have been added to the clinical activity, due to on-site production of Ga-68 from generators and the use of automated synthesis systems [9].

Such radiopharmaceuticals are manufactured or prepared in hospital radiopharmacies in accordance with the requirements of Good Manufacturing Practices (GMP) or Current Good Radiopharmacy Practice (cGRPP) [10-16].

Quality control of the prepared radiopharmaceuticals is necessary to characterize the final product relating to pharmaceutical parameters (to ensure that no microbiological, pyrogenic or particulate contamination can harm patients) and radioactivity measures designed to ensure minimum radiation exposure of patients by confirming high radiochemical and radio-nuclidic purity.

Indeed, impurities of the radionuclide and/or its chemical composition may affect the biodistribution of the injected radiopharmaceutical and consequently the radiation dose to any one particular organ or the whole body dose.

However, the lack of peer-reviewed quality control guidelines and recommendations for their application in human patients is a major concern. Therefore, it's necessary to apply general monographs in testing quality of prepared radiopharmaceuticals for clinical and research applications. 17 Acceptance criteria for quality control results require standardization and validation in labeling and preparation, including QC measurements to demonstrate that the procedure will reproducibly achieve its desired outcome [18].

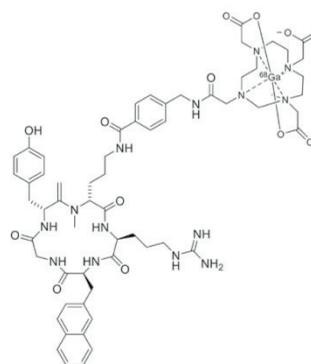


Figure 1. [^{68}Ga]-Pentixafor chemical structure

In this manuscript we report on the entire production cycle of [^{68}Ga]-DOTA-Pentixafor (Figure 1), including radiosynthesis with an automated synthesis system, quality control development and process validation [19-21].

Materials and Methods

Reagents

All reagents were high purity pharmaceutical grade. Hydrochloric acid solution (HCl 0.1 N) was obtained from Rotem GmbH.

The reagents trifluoroacetic acid, acetonitrile, ammonium acetate used for HPLC and ITLC phase, are purchased from Sigma Aldrich (Saint Louis, Missouri, USA) and are ultrapure or trace metal free.

The GMP-grade peptide Pentixafor, or DOTA-(Tyr3)-pentixafor was obtained from ABX pharmaceuticals (ABX, Advanced Biochemical Compounds, Radeberg, Germany) as a 1 mg powder which is dissolved in 1 mL of water and used in 10 μL aliquots.

The Pentixafor was obtained as a Good Manufacturing Process (GMP) grade product.

Automated module and synthesis cassettes

The synthesis of [^{68}Ga]-DOTA-Pentixafor is performed using a fully automated module (Scintomics GRP[®], Fuerstenfeldbruck, Germany) designed specifically for the synthesis of radiopharmaceuticals.

The module is controlled by a computerised system which runs the valves and syringes on the cassettes producing the desired radiopharmaceutical.

There is a specific cassette for the synthesis of [⁶⁸Ga]-DOTA-Pentixafor as well as for generator elution. The production cassettes, sterile and single-use, are obtained from ABX pharmaceuticals (ABX, Advanced Biochemical Compounds, Radeberg, Germany). The system is actually used for the production of other radiopharmaceuticals, such as [⁶⁸Ga]-PSMA, [⁶⁸Ga]-DOTATOC, [⁶⁸Ga]-DOTATATE, using a specific synthesis cassette. For each specific radiopharmaceutical process there is a specific template computer program.

Generator

The ⁶⁸Ge/⁶⁸Ga generator used in our Radiopharmacy is a Good Manufacturing Practice (GMP) compliant generator (GalliaPharm® Eckert & Ziegler Radiopharma GmbH, Germany).

The generator used was a 1.85 GBq. Elution is performed on the first day of the week and on non-synthesis days, due to the build-up of metal ions on the column requiring to be eluted on a daily basis and within 24 hours prior to any [⁶⁸Ga]-DOTA-Pentixafor synthesis. Elution process is performed using a 0.1 M HCl solution driven by the GRP™-Interface software in approximately five minutes. The elution volume is 10 mL.

Labeling procedure

Synthesis of [⁶⁸Ga]-DOTA-Pentixafor was performed using 10 µg of peptide.

Formula: C₆₀H₈₀N₁₄O₁₄ · x CH₃CO₂H

The synthesis process is shown in figure 2.

Packaging of the sterile synthesis cassette is removed in laminar flow hot cell model NMC 68-GA and all connections tightened. Then, the cassette is attached to synthesis module. Subsequently, we attach the empty containers, the syringe, the waste bottle, and reactor vial.

The reagents (0.9% saline, ethanol, eluent, and DOTA-pentixafor peptide in HEPES buffer) are

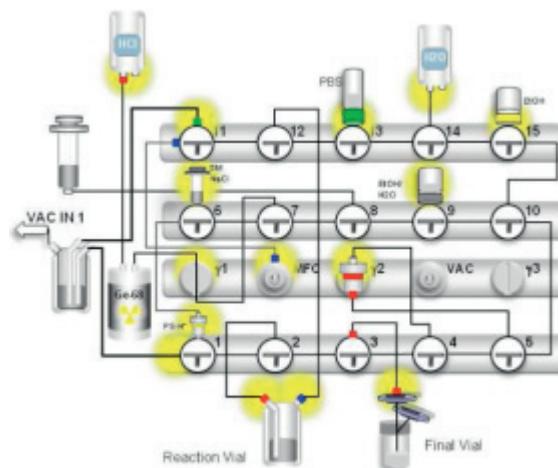


Figure 2. [⁶⁸Ga]-Pentixafor synthesis scheme

placed on the the synthesis cassette after closing all valves, and ⁶⁸Ge/⁶⁸Ga generator tube are connected.

⁶⁸GaCl₃ is eluted from ⁶⁸Ge/⁶⁸Ga generator with 10 ml of sterile ultrapure HCl 0,1M and collected to the pre-purification exchange cartridge, which is then eluted with NaCl 5M.

The Ga-68 chloride is released from the exchange column with 1,6 ml of NaCl 0,9% and added in the reaction vial containing DOTA-Pentixafor peptide (20 µg in 1.5 M 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (HEPES buffer solution) at pH = 4).

The mixture is incubated at 95 °C for 10 min. 18 After incubation the contents of the reaction vial are removed and pushed through the C₁₈ exchange cartridge (initially conditioned with ethanol, followed by water for injection Ph. Eur.) where the [⁶⁸Ga]-DOTA-Pentixafor is trapped; the unlabeled Ga-68 and DOTA-Pentixafor were collected to the waste.

The trapped [⁶⁸Ga]-DOTA-Pentixafor is recovered from the C18 column by 2 ml of Ethanol/Water 1/1.

The final product is diluted with 10 mL of phosphate buffered saline (PBS) and sterilized by filtration through an inline 0.22 µm sterile filter with a PVDF membrane.

At the end of the synthesis process a report is saved on the computer for the auditing trail.

The synthesis process takes approximately 33 minutes.

Analysis and Quality Control

The requirements for quality control of radiopharmaceuticals are listed in the European Pharmacopoeia. For typical analytical methods (e.g. analysis of residual solvents) used for QCs and characterization of [⁶⁸Ga]-DOTA-Pentixafor, ICH guidelines have been applied, while specific adjustments were performed for radioanalytical methods, such as radio-HPLC, radio-TLC and gamma spectrometry [22-24].

For the identification test the “pharmaceutical” part of the radiopharmaceutical was identified comparing the chromatographic retention time of the main radioactive peak and the “cold” standard; radionuclide identification was performed using gamma spectrometry analysis of the emitted energies.

All the analytical procedures were performed as defined in the standard operating procedures (SOPs) established in our Radiopharmacy Laboratory.

Appearance

The solution was visually analyzed per batch. The visual inspection of in-house prepared radiopharmaceuticals is necessary before injection to the patient being a measure of process performance and validation. Presence of particulate in the sample suggests possible failure during radiopharmaceutical synthesis, including purification, sterilizing filtration, and failed environmental control during the setting-up of reagents [25].

Instant Thin Layer Chromatography (ITLC)

The quality control of [⁶⁸Ga]-DOTA-Pentixafor was performed by Instant Thin Layer Chromatography (ITLC) and High Pressure Liquid Chromatography (HPLC), using the 0.2 mL sample taken directly from the final product.

The ITLC test was used to determine the radiochemical purity (RCP) of [⁶⁸Ga]-DOTA-Pentixafor and Ga-68 impurities as Ga-68 ion and Ga-68 colloid in the final product. The ITLC paper strips were counted in the gamma-counter (Cyclone^o Plus Storage Phosphor system, Perkin Elmer).

To measure the percentage of Ga-68 colloid, 1 mol/L ammonium acetate/methanol (1 : 1) / ITLC-SG strip (Varian iTLC-SG plates) is used: Ga-68 colloid (Rf= 0.0-0.2), Ga-68 peptide (Rf= 0.8-1) and Ga-68 ion (Rf=0.8-1).

High Pressure Liquid Chromatography (HPLC)

HPLC analyses were performed on a Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific) equipped with an AcclaimTM 120 C18 column 3µm 120Å (3.0 mm × 150 mm) and a UV and a -detector (Berthold Technologies, Milan, Italy). The used solvents were A) water +0.1% trifluoroacetic acid (TFA) and B) acetonitrile +0.1% TFA. HPLC eluents, water, acetonitrile, and trifluoroacetic acid (TFA) were of high-grade purity. The flow rate of the mobile phase was set at 0.6 mL/min, with a total run of 12 min.

The following gradient was used upon HPLC analysis: 0-2.5 min 15% B, 2.5-6 min from 15% B to 90% B, 6-8.01 min 90% B, 8.01-10 min 90% B to 15% B, 10-12 min 15% B.

The column temperature was established at 30°C. The samples were also monitored with UV detector at 220 nm for detection of chemical impurities. The software system Chromeleon 7 was used to put together the informations.

To prepare the reference “cold” standard of Pentixafor, we purchased the peptide from Scintomics GmbH.

Ge-68 Breakthrough

The Ge-68 breakthrough was determined in an elution sample, after a complete Ga-68 decay (>48 hours), on a weekly basis.

The γ-ray spectrometry tests included the identification of principal γ-photon (499–521 KeV peak) and ⁶⁸Ge content (decay of 499–521 KeV peak≥48 h) using a large volume counter linked to a multichannel analyser system (HPGe detector ORTEC GEM 30P4-76).

pH

The pH value of [⁶⁸Ga]-DOTA-Pentixafor was measured using pH strips (0-14) which show a

different colour at different pH range of the samples. The estimated value is registered.

Endotoxin and sterility

Quantitative determination of bacterial endotoxins was performed by the chromogenic method, using an Endosafe® *Portable Test System*-PTS. [⁶⁸Ga]-DOTA-Pentixafor samples were previously diluted and then applied in duplicate inside cartridges in parallel with positive control testing. The radiopharmaceutical was considered apyrogenic when the level of endotoxins was less than 17.5/V IU/mL.

The sterility of the [⁶⁸Ga]-DOTA-Pentixafor solution was assessed by direct inoculation of [⁶⁸Ga]-DOTA-Pentixafor solution in a growth broth which was incubated at 25 °C and 37 °C, respectively, and verified daily over fourteen days. The sample was considered sterile when there microbiological growth was absent.

Radionuclide identification and activity measurements

Radionuclidic purity was determined based on the half-lives, the type of emitted radiations and the energy of the radiation.

Ga-68 was measured with a dose calibrator (Capintec 25-R) in the eluate immediately after elution and in the radiopharmaceutical immediately after preparation. The γ -ray spectrometry tests included the identification of principal γ -photon (499–521 KeV peak) using a large volume counter linked to a multichannel analyser system (HPGe detector ORTEC GEM 30P4-76).

Half-life of ⁶⁸Ga was calculated after measuring the radioactivity of a sample in the dose calibrator at four consecutive intervals (5, 10, 15 and 20 minutes), then using the equation $T_{1/2} = \ln(1/2)/\lambda$, where λ = decay constant.

Results

the synthesis unit with labeled components is detailed in figure 3.



Figure 3. The automated synthesis unit

A total of 3 development radiosynthesis runs were analyzed and results are summarized in table 1.

The final formulation was [⁶⁸Ga]-DOTA-Pentixafor in a 16 mL solution of phosphate buffer saline (PBS). Properties of the acceptable product included a pH of 7, a clear colorless solution free of particles, <10% ethanol by volume (mean 5.37% V/V), and endotoxins <17.5 EU/ml. The total automated synthesis time was 33 minutes.

Ge-68 Breakthrough

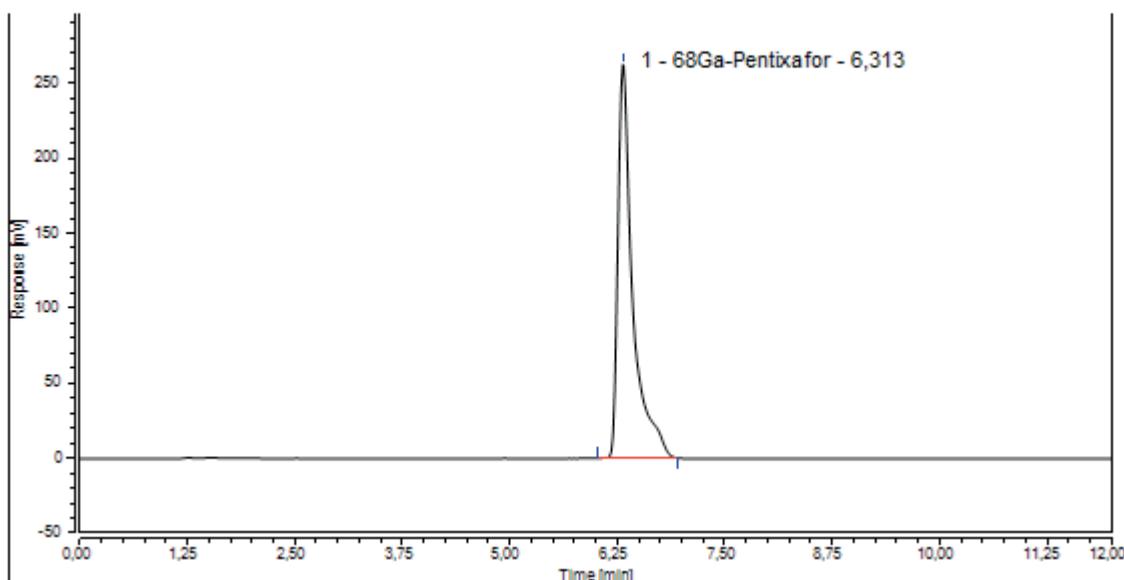
The Ge-68 breakthrough determined in the three samples was constantly far below the level recommended by *Pb.Eur.* (0.001% of the total radioactivity) and, respectively, $4.8 \cdot 10^{-5}$ %, $4.9 \cdot 10^{-5}$ % and $4.7 \cdot 10^{-5}$ %.

Radiochemical Quality Control

Assessment of radiochemical purity of the final product was performed using ITLC and HPLC. RCP as determined by HPLC in the three consecutive runs was 99.86%, 99.83% and 100%, respectively. Under the chromatographic conditions described above, [⁶⁸Ga]-DOTA-Pentixafor peak was well resolved; the average retention time was 6.1 min, as illustrated in typical chromatogram (figure 4). The radiochemical purity of this radiopharmaceutical was also confirmed with a TLC chromatography, which showed only the

Table 1. QC results of [⁶⁸Ga]-Pentixafor synthesis

Quality Control data	Run 1	Run 1	Run 3
Appearance	Colorless, no particles	Colorless, no particles	Colorless, no particles
Radionuclidic Identity	67.71 min	67.71 min	67.71 min
Radionuclidic Purity	100,00%	100,00%	100,00%
Radiochemical Identity	RT = 6.313 min	RT = 6.360 min	RT = 6.351 min
Radiochemical Purity	99.86%	99,83% %	100%
Chemical Purity	compliant	compliant	compliant
⁶⁸ Ge Impurity	0.0000041%	0.0000041%	0.0000041%
pH	7	7	7
LAL Test	<17.5 EU/ml	<17.5 EU/ml	<17.5 EU/ml
Residual EtOH content	5.22% V/V	5.58% V/V	5.32% V/V
Yield (%)	56.7	56.9	57.3
Activity (MBq)	459 MBq	461 MBq	464 MBq

**Figure 4.** HPLC traces of [⁶⁸Ga]-Pentixafor

presence of [⁶⁸Ga]-DOTA-Pentixafor at a retention time of 0.8-1 (figure 5) in all batches.

Chemical purity

A chemical impurity which has been verified by gas chromatography is the presence of residual ethanol. As it shown in the chromatogram (figure 6), the

peak related to the presence of ethanol is at a retention time of 2.10 minutes; the amount of EtOH was <10% in all batches (respectively 5.22%, 5.58% and 5.32% V/V).

Subsequently, we also evaluated the residual quantity of HEPES in the final preparation by TLC, that was less intense than the spot corresponding to the reference solution according to *Ph.Eur.* (200 µg/V).

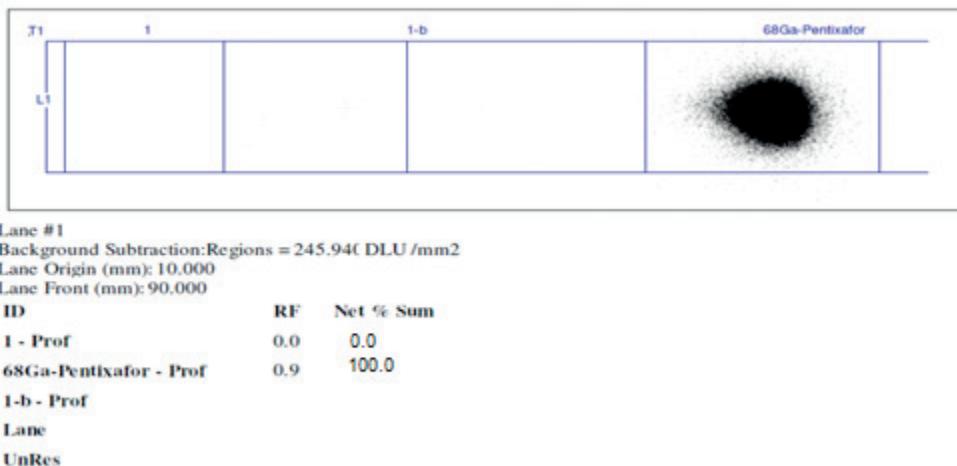


Figure 5. TLC of [⁶⁸Ga]-Pentixafor

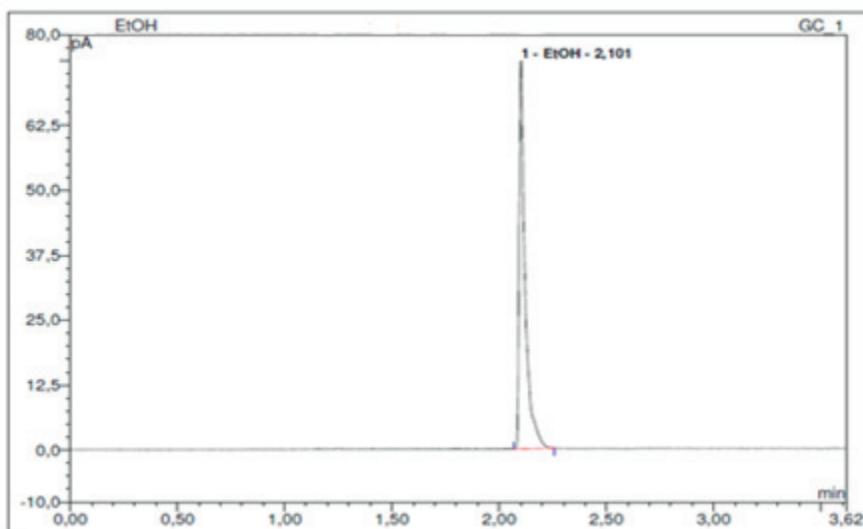


Figure 6. Gas chromatography analysis of [⁶⁸Ga]-DOTA-Pentixafor

Radionuclide identification

The spectrum obtained by gamma spectroscopy showed peaks at energies equal to 0.511 MeV and 1.077 MeV corresponding to Ga-68.

It was also confirmed by the calculation of the half-life of Ga-68 with the formula:

$$t_{1/2} = 0.693 t / (\ln A_0 / A_t)$$

Approximate half-life of Ga-68 was 67.71 min.

pH and sterility

The pH was 7 in all the validation runs and LAL test for bacterial endotoxin resulted <17.5 EU/ml in all the three synthesis samples.

Reproducibility

The activity of the final product (Ga-68 labeled DOTA-Pentixafor) depends on the Ga-68 activity

eluted from the $^{68}\text{Ge}/^{68}\text{Ga}$ generator. From the eluate ($^{68}\text{GaCl}_3$) of our generator of 810 ± 10 MBq the automated synthesis module can produce 450–480 MBq of [^{68}Ga]-DOTA-Pentixafor.

The total production time of Ga-68 labeled peptide was ~45 min including initial setup and all QC testing needed for the release. The mean yield in the validation runs was 57% and the mean radiochemical purity was 99.90%.

Discussion and Conclusion

The continuing development of new tools for molecular imaging is not accompanied by a coherent effort in development, standardization, and validation of quality control methods to guarantee high-quality radiopharmaceutical production, especially in the routine clinical setting. Validation of the procedures used in the radiopharmacy is therefore the means for providing assurance that products will be of the required quality.

During years we have set up a few SPECT and PET tracers for research or clinical purpose [21], therefore it became mandatory to define a validation master plan, covering all the intended validation / qualification activities for radiopharmaceutical preparations, according to the existing guidelines [26–29].

The increasing number of different radiopharmaceuticals creates challenges for the production process at radiopharmacies. Recent commercial automated synthesis systems based on disposable cassettes for each radiolabeled probe simplify the production of multiple tracers avoiding the need of tracer-specific customization. A major advantage of an automated system over manual synthesis is the increased reproducibility by reducing operator variability [30–32]. These systems enabling the operator to develop and optimize synthesis protocols of radiopharmaceuticals to achieve the desired yield and purity. Then, a validation of the synthesis process has to be performed for the production of clinical-grade tracers.

This is particularly important in clinical use, both with respect to GMP compliance and for maintaining

the high specific activity requirement for accurate PET quantitation.

Our validation runs demonstrated highly reproducible synthesis yields (mean value 57%).

The radiochemical purity of [^{68}Ga]-DOTA-Pentixafor was measured using both ITLC and HPLC techniques and results showed that the synthesized product was well above the recommended level (99.90%) in all the validation runs.

The high radiochemical purity of the final product demonstrated a consistent quality production system that guarantees patient safety, image quality and accurate quantification of tracer accumulation.

The next goal will be the development and validation of the high-pressure liquid chromatography method for the determination of chemical purity and radiochemical purity of [^{68}Ga]-DOTA-Pentixafor.

List of Abbreviations

CXCR4: Chemokine receptor 4

DOTA: 1.4.7.10- tetraazacyclododecane-1,4,7,10-tetraacetic acid

GMP: Good Manufacturing Practices

GC: gas chromatography

GRP: Good Radiopharmaceutical Practices

HPLC: High Pressure Liquid Chromatography

ITLC: Instant Thin Layer Chromatography

LAL: Limulus Amebocyte Lysate

PET: Positron Emission Tomography

QC: Quality Control

RCP: Radiochemical Purity

RCY: Radiochemical yield

TFA: Trifluoroacetic Acid

Authors' Contributions: AS and SM carried out the synthesis, validation and quality controls procedures, participated in the data analysis and drafted the manuscript. AS and LR conceived and design the study. MS and GB participated in its design and coordination and helped to draft the manuscript. LR supervised the study and revised the manuscript.

Authors' Information: Authors of this paper are competent in many different disciplines from basic science to clinic. Radiopharmaceutical based procedures are complex and require a multidisci-

plinary approach with different skills related to radiochemistry (AS, SM), biochemistry (AS), nuclear medicine (GB, MS, LR).

All authors read and approved the final manuscript.

Authors' Contributions: Antonino Sammartano, Biologist: ansammartano@ao.pr.it; Silvia Migliari, Radiopharmacist: smigliari@ao.pr.it; Giorgio Baldari, MD: gbaldari@ao.pr.it; Maura Scarlattei, MD: mscarlattei@ao.pr.it; Livia Ruffini, MD, Head of the Department: lruffini@ao.pr.it; Synthesis and CQ of [68Ga]-DOTA-Pentixafor

References

- Buck AK, Stolzenburg A, Hänscheid H, Schirbel A, Lück-erath K, Schottelius M, Wester HJ, Lapa C. Chemokine receptor - Directed imaging and therapy. *Methods*. 2017 Nov 1;130:63–71. doi: 10.1016/j.ymeth.2017.09.002. Epub 2017 Sep 12. Review.
- Burger JA and Kipps TJ. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. *Blood*. 2006; 107:1761–1767.
- Burger JA and Peled A. CXCR4 antagonists: targeting the microenvironment in leukemia and other cancers. *Leukemia*. 2009; 23:43–52.
- Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E and Zlotnik A. Involvement of chemokine receptors in breast cancer metastasis. *Nature*. 2001; 410:50–56.
- Zhang B, Wu T, Wang Z, Zhang Y, Wang J, Yang B, Zhao Y, Rao Z and Gao J. p38MAPK activation mediates tumor necrosis factor- α -induced apoptosis in glioma cells. *Molecular medicine reports*. 2015; 11:3101–3107.
- Kaemmerer D, Reimann C, Specht E, Wirtz RM, Sayeg M, Baum RP, Schulz S and Lupp A. Differential expression and prognostic value of the chemokine receptor CXCR4 in bronchopulmonary neuroendocrine neoplasms. *Oncotarget*. 2015; 6:3346–3358. doi: 10.18632/oncotarget.3242.
- Andreas Johannes Poschenrieder. Development of Diagnostic and Therapeutic Radiopharmaceuticals targeting the Chemokine Receptor 4. <https://d-nb.info/1142376478/34>
- Poschenrieder, A., Schottelius, M., Schwaiger, M. *et al*. Preclinical evaluation of [⁶⁸Ga]NOTA-pentixafor for PET imaging of CXCR4 expression in vivo — a comparison to [⁶⁸Ga]pentixafor. *EJNMMI Res* 6, 70 (2016) doi:10.1186/s13550-016-0227-2
- A. Sammartano, S. Migliari, M. Scarlattei, G. Baldari, L. Ruffini. Synthesis and Quality Control of a new probe [68Ga]- Pentixafor for PET imaging of CXCR4 expression. <https://link.springer.com/content/pdf/10.1007%2F978-94-007-5901-8-3.pdf>
- Eur J Nucl Med Mol Imaging (2018) 45(Suppl 1): 1. <https://doi.org/10.1007/s00259-018-4148-3>
- Guidelines on current good Radio pharmacy Practice (cGRPP) in the Preparation of Radiopharmaceuticals. European Association of Nuclear Medicine. (2007). Version 2., http://www.eanm.org/publications/guidelines/gl_radio-ph_cgrpp.pdf.
- Elsinga P, Todde S, Penuelas I, Meyer G, Farstad B, Faivre-Chauvet A, Mikolajczak R, Westera G, Gmeiner-Stopar T, Decristoforo C. Guidance on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals. *Radiopharmacy Committee of the EANM. Eur J Nucl Med Mol Imaging*. 2010 May;37(5):1049–62
- International atomic energy agency. Quality control bin the Production of Radiopharmaceuticals. IAEA-TECDOC-1856 <https://www-pub.iaea.org/MTCD/Publications/PDF/TE-1856web.pdf>
- World Health Organization. WHO Expert Committee on Specifications for Pharmaceutical Preparations-WHO Technical Report Series, 908-Thirtyseventh Report., (2003). http://whqlibdoc.who.int/trs/who_trs_908.pdf.
- Kung-Tien Liu, Jian-Hua Zhao, Lee-Chung Men and Chien-Hsin Chen. Quality by Design and Risk Assessment for Radiopharmaceutical Manufacturing and Clinical Imaging. Chapter 13 <http://dx.doi.org/10.5772/51112>
- Barbet, J., Kraeber-Bodéré, F., & Chatal, J. F. Review: What Can Be Expected from Nuclear Medicine Tomorrow? *Cancer biotherapy and radiopharmaceuticals*, (2008). 23(4), 483–504
- Blois, E., Zanger, R.M.S., Chan, H.S. *et al*. Radiochemical and analytical aspects of inter-institutional quality control measurements on radiopharmaceuticals. *EJNMMI radiopharm. chem.* 4, 3 (2019)
- Decristoforo C, Penuelas I, Patt M, Todde S. European regulations for the introduction of novel radiopharmaceuticals in the clinical setting. *Q J Nucl Med Mol Imaging*. 2017 Jun;61(2):135–144.
- Todde S, Peitl PK, Elsinga P, Koziorowski J, Ferrari V, Ocak EM, Hjelstuen O, Patt M, Mindt TL, Behe M. Guidance on validation and qualification of processes and operations involving radiopharmaceuticals. *EJNMMI Radiopharm Chem*. 2017;2(1):8
- Aslani A, Snowdon GM, Bailey DL, Schembri GP, Bailey EA, Roach PJ. Gallium-68 DOTATATE Production with Automated PET Radiopharmaceutical Synthesis System: A Three Year Experience. *Asia Ocean J Nucl Med Biol*. 2014;2(2):75–86.
- Migliari S, Sammartano A, Scarlattei M, Serreli G, Ghetti C, Cidda C, Baldari G, Ortenzia O, Ruffini L. Development and Validation of a High-Pressure Liquid Chromatography Method for the Determination of Chemical Purity and Radiochemical Purity of a [(68)Ga]-Labeled Glu-Urea-Lys(Ahx)-HBED-CC (Positron Emission Tomography) Tracer. *ACS Omega*. 2017 Oct 31;2(10):7120–7126.
- ICH Q2(R1) Validation of Analytical Procedures: Text and Methodology - International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) <https://www.ich.org/fileadmin/>

- Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf
23. Annex 15 (EU) of Good Manufacturing Practice (GMP) guidelines: EDQM Guidelines: Guide for the elaboration of monographs on RADIOPHARMACEUTICAL PREPARATIONS European Pharmacopoeia Edition 2018
 24. European Pharmacopoeia, 9th Edition, volume 1: chapter 2.2.66; Detection and measurement of radioactivity, 01/2017.
 25. Shukla J, Vatsa R, Garg N, Bhusari P, Watts A, Mittal BR. Quality control of positron emission tomography radiopharmaceuticals: An institutional experience. *Indian J Nucl Med.* 2013 Oct;28(4):200–6.
 26. Migliari S, Sammartano A, Cidda C, Baldari G, Scarlattei M, Serreli G, Ghetti C, Pipitone S, Lippi G, Ruffini L. Novel approach for quality assessment and improving diagnostic accuracy in cell-based infection imaging using ^{99m}Tc -HMPAO labeled leukocytes. *Acta Biomed.* 2018 Oct 8;89(3):355–364. doi: 10.23750/abm.v89i3.7064. PubMed PMID: 30333459.
 27. Sammartano, A.; Scarlattei, M.; Migliari, S.; Baldari, G.; Ruffini, L. Validation of in Vitro Labeling Method for Human Use of Heat-Damage Red Blood Cells to Detect Splenic Tissue and Hemocateretic Function. *Acta Bio Med* 2019, 90, 275–280
 28. Migliari S, Sammartano A, Scarlattei M, Serreli G, Ghetti C, Cidda C, et al. Development and Validation of a High-Pressure Liquid Chromatography Method for the Determination of Chemical Purity and Radiochemical Purity of a [^{68}Ga]-Labeled Glu-Urea-Lys(Ahx)-HBEDCC (Positron Emission Tomography) Tracer. *ACS Omega.* American Chemical Society; 2017 Oct 31;2(10):7120–6.
 29. Andrea Sartori, Francesca Bianchini, Silvia Migliari, Paola Burreddu, Claudio Curti, Federica Vacondio, Daniela Arosio, Livia Ruffini, Gloria Rassu, Lido Calorini, Alberto Pupi, Franca Zanardi and Lucia Battistini. Synthesis and Preclinical Evaluation of a Novel, Selective ^{111}In -labelled Aminoproline-RGD-peptide For Non-invasive Melanoma Tumor Imaging. *Med. Chem. Commun.*, 2015,6, 2175–2183
 30. Decker MD and Turner JH. Automated Module Radiolabeling of Peptides and Antibodies with Gallium-68, Lutetium-177 and Iodine-131. *Cancer Biother Radiopharm* 2012; 27: 72–76
 31. Decristoforo C, Knopp R, von Guggenberg E, Rupprich M, Dreger T, Hess A, Virgolini I and Haubner R. A fully automated synthesis for the preparation of ^{68}Ga -labelled peptides. *Nucl Med Commun* 2007; 28: 870–875.
 32. Ocak M, Antretter M, Knopp R, Kunkel F, Petrik M, Bergsadi N and Decristoforo C. Full automation of ^{68}Ga labelling of DOTA-peptides including cation exchange prepurification. *Appl Radiat Isot* 2010; 68: 297–302.

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Correspondence:

Antonino Sammartano

Nuclear Medicine and Molecular Imaging Department, Azienda Ospedaliero-Universitaria di Parma, Italy

Telephone: 0039 3317867654

E-mail: ansammartano@ao.pr.it