Received: 20 June 2023 | Revised: 17 August 2023 | Accepted: 18 August 2023

DOI: 10.1002/jlcr.4061

RESEARCH ARTICLE

Journal of Labelled Compounds and Radiopharmaceuticals WILEY

[^{68}Ga]Ga-PentixaFor: Development of a fully automated in hospital production on the Trasis miniAllinOne synthesizer

Julien Costes[1] | **Kilian Casasagrande**[1] | **Constance Dubegny**[2] | **Juan Castillo**[3] | **Jens Kaufman**[3] | **Julien Masset**[4] | **Charles Vriamont**[4] | **Corentin Warnier**[4] | **Alain Faivre-Chauvet**[5] | **Judith Anna Delage**[1]

[1]Radiopharmacy Unit, Department of Pharmacy, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland

[2]Radiopharmacy Unit, Department of Pharmacy, Nantes University Hospital, Nantes, France

[3]Pentixapharm GmbH, Berlin, Germany

[4]Department of Research and Development, Trasis Radiopharmacy Instruments, Ans, Belgium

[5]Nantes University, Nantes University Hospital, Inserm UMR 1307, CNRS UMR 6075, CRCI2NA, Nantes, France

Correspondence
Judith Anna Delage, Radiopharmacy Unit, Department of Pharmacy, Lausanne University Hospital and University of Lausanne, CH-1011, Lausanne, Switzerland.
Email: judith.delage@chuv.ch

Funding information
This research did not receive any specific grant from funding agencies in the public, commercial, or not profit sectors.

[^{68}Ga]Ga-PentixaFor is a frequently used radiotracer to image the CXCR4/CXCL12 axis in various malignancies, infections, and cardiovascular diseases. To answer increasing clinical needs, an automatized synthesis process ensuring efficient and reproducible production and improving operator's radioprotection is needed. [^{68}Ga]Ga-PentixaFor synthesis has been described on other synthesizers but not on the miniAiO. In this work, we defined automated synthesis process and an analytical method for the quality control of [^{68}Ga]Ga-PentixaFor. Validation batches were performed under aseptic conditions in a class A hotcell. All the quality controls required by the European Pharmacopea (Eur. Ph) were performed. The analytical methods were validated according to the International Conference Harmonization (ICH) recommendations. Validation batches were performed with a radiochemical yield of 94.8 ± 2.6%. All the quality controls were in conformity with the Eur. Ph, and the validation of the analytical method complied with the ICH. The environmental monitoring performed during the synthesis process showed that the aseptic conditions were ensured. [^{68}Ga]Ga-PentixaFor was successfully synthesized with the miniAiO by a fully automated process. This robust production mode and the quality control have been validated in this study allowing to increase the access of patients to this new promising radiopharmaceutical.

KEYWORDS
[^{68}Ga]Ga-PentixaFor, automated production, miniAllinOne, quality control, radiopharmaceutical, validation method

1 | INTRODUCTION

CXCR4 is a chemokine receptor belonging to the 7-transmembrane G protein-coupled receptor that plays a major role in the communication pathways. CXCR4 has a high expression level in lymphocytes as well as hematopoietic stem cells, stromal fibroblast, cancer cells, endothelial, and epithelial stem cells.[1]

This receptor binds to its endogenous ligand CXCL12. The CXCR4/CXCL12 axis is involved in many

physiological process like organogenesis, angiogenesis, immune cell trafficking, and hematopoiesis[2] but plays also a major role in pathological conditions. In these processes, the central functional element is the migration of CXCR4-expressing cells towards tissues with high local CXCL12 expression and their retention and differentiation at these sites.[3,4]

CXCR4 role has been reported in various solid or hematological tumor diseases. Its upregulation has been identified as a key factor in tumor cells survival, proliferation, migration, and metastasis.[5] Additionally, to oncology, CXCR4/CXCL12 acts in the inflammatory process, and its overexpression is involved in autoimmune diseases. Indeed, the CXCR4-CXCL12 axis also plays a major role in the homing and mobilization of leucocytes.[6,7]

CXCR4 is an interesting target that can be used with promising results in both imaging[8] and therapy[9] of oncological or inflammatory diseases. Development of radiopharmaceuticals for positron emission tomography (PET) had thus been performed. In 2011, Demmer et al. were the first to synthetize CXCR4-binding peptide for molecular imaging.[10] Since, three classes of imaging agent have been developed for PET imaging of CXCR4 expression in humans.[11] Among them were the radiolabeled analogs of the bicyclams AMD3100 and AMD3465, the ^{18}F- or ^{68}Ga-labeled T-140-based peptides, and the radiolabeled, FC-131-based cyclic pentapeptides. Representatives of the first two groups generally display high splenic and hepatic uptake in mice and humans, challenging their suitability for high contrast imaging purposes. However, one representative of the last group, the small cyclic pentapeptide [^{68}Ga]Ga-PentixaFor, demonstrates affinity and specificity for CXCR4 with a fast renal elimination and very low non-specific background accumulation, giving a high imaging contrast of tissue with CXCR4 expression in humans using PET.[5]

Therefore, [^{68}Ga]Ga-PentixaFor is now a frequently used radiotracer to image the CXCR4/CXCL12 axis in various malignancies, infections and cardiovascular diseases.[12] The absorbed dose in healthy organs is lower than other PET radiopharmaceuticals and is well tolerated.[13]

The radiolabeling of [^{68}Ga]Ga-PentixaFor is performed with a DOTA chelator. This radiolabeled compound provides a high affinity to the CXCR4 receptor.[10] To answer to clinical needs of upcoming clinical studies, ensure a reproducibility of the process, and improve operator's radioprotection, a safe and efficient production via an automated module process is desirable.

So far, the automated synthesis of [^{68}Ga]Ga-PentixaFor has already been described on Scintomics[14,15] and Eckert and Ziegler[16] modules, but not on the miniAllinOne (miniAiO) from Trasis.

Herein, we describe a simplified, good manufacturing practices (GMP) compliant automated synthesis process by using a ^{68}Ge/^{68}Ga generator (GalliaPharm) and the miniAiO synthesizer as well as analytical method validation for the quality control of this product.

2 | EXPERIMENTAL

2.1 | Automated production of [^{68}Ga]Ga-PentixaFor

The synthesis process was validated on two production sites (University Hospitals of Nantes and Lausanne) by performing standardized validation batches as follows. The GMP grade Pentixafor acetate, DOTA conjugated, was provided by PentixaPharm (Würzburg, Germany) in lyophilized vials containing 50 µg of net peptide. The structure of the precursor is detailed in Figure 1.

The GMP grade ^{68}Ge/^{68}Ga generator Galliapharm, 1850 MBq, was supplied by Eckert & Ziegler Radiopharma GmBH (Berlin, Germany). The miniAiO synthesizer, the disposable cassette, and the reagents set used for the synthesis were provided by Trasis (Ans, Belgium). The reagent set contained a vial of 5 mL of ethanol Emsure grade, a bag of 50 mL of NaCl 0.9%, and a syringe filled with 1 mL of 0.7 M sodium acetate traceselect

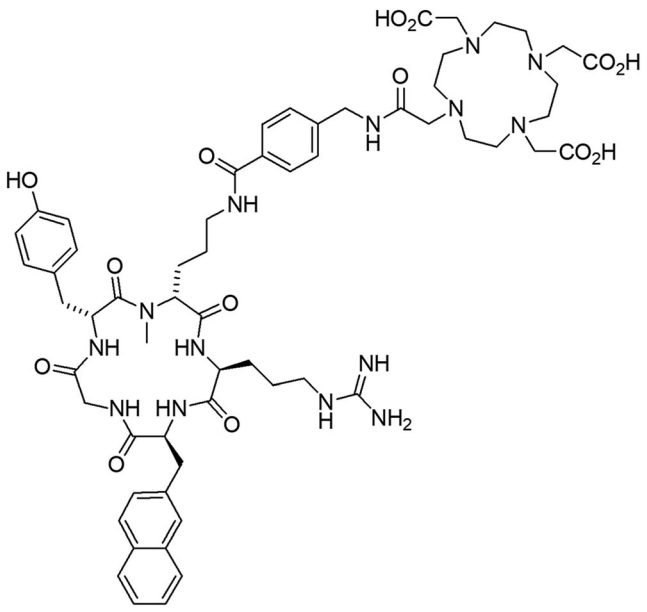

FIGURE 1 Chemical structure of the Pentixafor (supplied as an acetate salt). The compound contains one DOTA chelator for the complexation with ^{68}Ga. Acetate counterions are omitted for clarity.

buffer. A hydrophilic/hydrophobic (modified acrylic and polyethersulfone membrane) non-vented 0.22 μm sterile filter was used. The sterile eluent was HCl 0.1 N for ultra-trace analysis. All the reagents and cassettes were low bioburden and endotoxins.

The synthesizer was first equipped with the single use cassette. The ethanol and the NaCl 0.9% contained in the reagents set were directly connected to the cassette. The synthesis process was performed with a fully automated process controlled by a software (Trasis, Ans, Belgium) without any manual intervention, while the synthesis template was setup on the available software. The peptide was solubilized with 1 mL of sodium acetate, and the peptide solution was transferred into the reaction vial.

The radio labelling was performed by eluting the $^{68}Ge/^{68}Ga$ generator with 5 mL of HCl 0.1 N into the reaction vial. No pre-purification of the eluate was needed. The reaction took place at pH 4.0, and the heating block starting temperature was set to 120°C during 1.5 min, followed by 8.5 min at 95°C. The temperature in the reaction vial was monitored by a calibrated probe. This ensures that the actual temperature in the reaction solution remains at 95°C for the whole 10 min of the radiolabeling reaction. The reaction solution was subsequently transferred to an apolar HLB (hydrophilic–lipophilic balance) cartridge for purification. After rinsing the reactor and removing hydrophilic reaction impurities by passing 10 mL of 0.9% NaCl on the HLB cartridge, the final product was eluted with 0.7 mL of ethanol and transferred to the final sterile vial through the 0.22 μm filter. The solution was finally formulated with filtered NaCl 0.9% to a final volume of 10 mL and measured with a dose calibrator (Veenstra VDC 405 Comecer, Castel Bolognese, Italy; CRC-55tR, Capintec, Florham Park, NJ, United States).

As conventional radiopharmaceuticals, all the batches were prepared in accordance with the requirements of the Good Manufactural Practices (GMP) or the Current Good Radiopharmacy Practice (cGRPP).[17,18]

2.2 | Analytical procedures

For each batch, all the quality controls required by the European Pharmacopeia (Eur. Ph) were done.

2.2.1 | Filter integrity test

A filter integrity test was performed automatically at the end of synthesis by the miniAiO synthesizer through a holding pressure test. The minimum value is set by the provider of the filter: 2.5 bar minimum.

2.2.2 | Radiochemical yield (RCY)

At the end of synthesis, the final activity was measured for the validation batches and the RCY was calculated for each one. The mean RCY obtained in Lausanne and Nantes were compared.

2.2.3 | Chromatography analyses

High performance liquid chromatography (HPLC) analyses were done using different devices in function of the site of production: an Ultimate 3000 SD System (Thermo Fisher Scientific, Waltham, MA, USA) or an Breeze QS system (Waters corporation, Milford, Massachusetts, USA) and a GabiStar radiodetector (Elysia-Raytest GmBH, Straubenhard, Germany) or a F-Lumo radiodetector (Berthold, Dieue sur Meuse, France) for Lausanne and Nantes sites, respectively. Compounds were separated with a Gemini column, 3 μm, peptide NX-C18, 100 Å, dimension 4.5 × 150 mm (Phenomenex, Torrance, CA, USA). The mobile phase was composed of A: H_2O/TFA, 100/0.1 (V/V) and B: Acetonitrile/TFA, 100/0.1. The injection volume was 20 μL, and the sequence was run during 15 min with the following gradient: 0–9.0 min, A = 80% to 50%; 9–10 min, A = 50%; 10.0–11.0 min, A = 50% to 80%; 11.0–15.0 min, A = 80%. The wavelength set up for the UV detection was 220 nm, and the range of energy for the gamma detection was 400–600 keV. All solvents were HPLC grade.

The thin-layer chromatography (TLC) scanner used were MiniGita (Raytest, GmBH, Straubenhard, Germany). The radio TLC was performed with the method described in the Eur. Ph. Ammonium acetate 1 M Reagent grade (Merck, Zug, Switzerland; Honeywell, Wabash, Indiana, USA) and methanol (Merck, Zug, Switzerland; VWR, Radnor, Pennsylvanie, USA) 50/50 (V/V) were used as mobile phase and ITLC-SG strips (Agilent Technologies, Folsom, CA, USA) were used as stationary phase.

The radiochemical purity (RCP) and the stability of the final product after end of synthesis were evaluated by radio-HPLC and TLC at 1 h and 2 h for both sites, and a complementary time point was added at 3 h for Lausanne. The mean RCP obtained with HPLC and TLC in Nantes and Lausanne was compared.

The identification of the final product was performed by HPLC comparison of the relative retention time of the compounds [^{68}Ga]Ga-PentixaFor and the reference standard [^{nat}Ga]Ga-Pentixafor (ABX Advanced Biochemical Compounds, Radeberg, Germany). The results presented are from Lausanne.

The peptide contained in the final product was quantified by UV HPLC using the linear regression obtained by the linearity of the UV detector. The quantification was performed with the data from Lausanne. The maximum quantity of peptide was determined to be 50 μg.

2.2.4 | Assessment of pH

The pH was determined by pH strips in Lausanne and Nantes. The pH of the final compounded radiopharmaceutical preparation shall be checked to ensure that its value is within the acceptable range resulting from the manufacturing process and suitable for IV administration.

2.2.5 | Assessment of residual solvent

The residual ethanol was measured by gas chromatography (GC) according to the Eur. Ph., withdrawing of the sample by an Autosampler in the GC 6850 system (Agilent Technologies, Santa Clara, USA). The mean residual ethanol obtained by Nantes and Lausanne was compared.

2.2.6 | ^{68}Ge breakthrough

The ^{68}Ge breakthrough was assessed in Lausanne with a gamma counter (AMG Automatic Gamma Counter, Hidex, Turku, Finland) at least 48 h after elution. The test consisted in measuring the residual activity of an elution, which reflect the ^{68}Ge breakthrough, and to calculate the ratio between this activity and the activity at EOE (end of elution) relative to ^{68}Ga.

2.3 | Validation of analytical procedure

The validation of the procedure was done in Lausanne.

2.3.1 | System suitability test (SST)

SST was performed to validate the identification of the final product and to assess the repeatability of the HPLC analysis. The substance [^{nat}Ga]Ga-Pentixafor acetate (PentixaPharm, Würzburg, Germany) was used as reference standard. The identification was done by determination of the retention time and of the symmetry factor of the reference (net peptide concentration 0.1 mg/mL). Net peptide (100 μg) was solubilized with 1 mL of water for injection. Five injections of 20 μL were applied to evaluate the repeatability of the system by calculation of the coefficient of variation. Retention times, peak areas, and symmetry factors were determined for each injection. The relative retention times (RRT) between [^{nat}Ga]Ga-Pentixafor and [^{68}Ga]Ga-PentixaFor were calculated for all the validation batches. The precision of this calculation was assessed by determining the coefficient of variation (CV). The value had to be less than 5.0%.

2.3.2 | Linearity

The linearity of the radioactive detector (RAD) was carried out with a solution of [^{68}Ga]$GaCl_3$, which was injected at decreasing activities in the HPLC. For each activity, the peak area was measured.

The linearity of the UV detector was performed by injection of increasing concentrations of [^{nat}Ga]Ga-Pentixafor acetate.

The limit of quantification that corresponds to the last injection with a signal/noise (S/N) ratio ≥ 10 was also determined.

2.3.3 | Accuracy

The accuracy test, which allows to exclude underestimation of impurities by irreversible retention in the column, was performed by the method of activity balances. The analysis was carried out in triplicate by injection of 20 μL of either the drug product (DP) or a standard in the HPLC. The solvent eluted was collected at the end of the column. All collected volumes were determined by weighing method. Aliquots of DP and standard solution were counted in the HIDEX gamma counter. After decay correction, the volumetric activities measured for aliquots were compared to the standard and the deviation was calculated. The standard error calculated had to be less than 5%.

2.3.4 | Radio TLC validation

The reference solution A of colloidal ^{68}Ga was prepared according to the prescriptions of Eur. Ph 01/2013:2482. This solution was eluted on the TLC, and the retardation factor (Rf) was determined by the scintillation detector.

The reference solution B of [^{68}Ga]Ga-Pentetate was prepared according to the monography 1/2013:2482 of the Eur. Ph. The solution was eluted by TLC, and the Rf was determined.

The solution of [^{68}Ga]Ga-PentixaFor was eluted with this method. The Rf obtained had to be between 0.8 and 1.0.

2.3.5 | pH test validation

pH strips were validated as followed: Merck brand pH strips 0.0–6.0 and 5.0–10.0 were humected with pH buffer 7.0, pH buffer 4.0, or drug product (DP) solution. One minute later, the strips were compared with the color scale.

2.4 | Microbiology validation

Sterility tests were performed on the validation batches. Three aliquots of 1 and 2 mL for Lausanne and Nantes, respectively, of the drug product were tested by a GMP certified laboratory in accordance with the Eur. Ph. (Quality Control Laboratory, Pharmacy department, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; Abteilung Mikrobiologie & Chemische Analytik, company: CUP Laboratorien, Radeberg, Germany).

Low bioburden level of disposable cassette, chemicals, and peptide was tested in Lausanne with simulating labelling according to the Eur. Ph. ^{68}Ge/^{68}Ga elution was replaced by a sterile NaCl 0.9% solution, and the 0.22 µm filter was removed.

During the entire synthesis process, environment was monitored by settle-plates. At the end of synthesis, the hands of the operator were checked for microbiological contamination by agar contact plate.

The endotoxin testing was performed by LAL assay according to the Eur. Ph. using Nexgen PTS device (Charles River, Wilmington, MA, USA). The sample was diluted to 1/40 and 1/100 in Lausanne and Nantes, respectively, to avoid enzymatic inhibition or induction of the LAL reaction.

2.5 | Statistics

Data from linearity were analyzed using Pearson correlation. For each concentration of either [^{68}Ga]$GaCl_3$ or [^{nat}Ga]Ga-Pentixafor acetate, respectively, for RAD or UV detector linearity, the peak area was measured, and the Pearson correlation coefficient between the concentrations and the peak areas was determined.

Significant differences between means were analyzed by an unpaired, two-tailed Student's t test with a correction for multiple comparison using the Holm-Sidak method ($p = 0.05$).

3 | RESULTS

3.1 | Validation of analytical method

The specifications and the results are summarized in Table 1.

3.1.1 | SST

The retention time obtained for [^{nat}Ga]Ga-Pentixafor was 4.93 ± 0.004 min ($n = 5$), the peak area was 160.4 ± 1.00 mAU·min ($n = 5$), and the symmetry factor was 0.85 ± 0.01 ($n = 5$). The coefficients of variation calculated for retention times, peak areas, and symmetry factor were, respectively, 0.1%, 0.6%, and 0.6%.

TABLE 1 Summary of the specifications and results for the validation of the analytical method (CV = coefficient of variation).

Parameters	Channel	Criteria	Specifications[a]	Results
System suitability test	UV	Retention times	CV ≤ 1.0%	0.1%
		Peak areas	CV ≤ 1.0%	0.6%
		Symmetry factor (CV)	0.8–1.8	0.85 (0.6%)
Identity	UV, RAD	RRT	0.95–1.05	0.995
Linearity	UV	R^2 value	$R^2 \geq 0.99$	0.9937
	RAD			0.9999
Precision	UV	Repeatability of RRT	CV ≤ 5.0%	0.2%
Accuracy	RAD	Standard error	≤5.0%	−3.6%

Note: The specifications of the parameters are defined accordingly to the International Conference of Harmonization (ICH).
[a]Specifications were set up according to the European Pharmacopeia 11th Edition.

3.1.2 | Linearity

For the linearity of the RAD detector, the R^2 obtained for the RAD detector was 0.9999 (Figure 2). The limit of quantification for this detector was 0.04 MBq/mL with a S/N ratio of 11.3.

The R^2 calculated for the UV detector was 0.9937, and the curve is represented in Figure 3. The limit of quantification obtained was 0.13 μg/mL (S/N ratio = 13.9).

3.1.3 | Accuracy test

The accuracy test was performed and the mean standard error was −3.6 ± 0.2% ($n = 3$).

3.1.4 | RadioTLC validation

The Rf obtained (Rf = 0.1) with the solution A was in accordance with the specifications (Rf ≤ 0.1).

The Rf obtained (Rf = 0.9) with the solution B was in conformity with the specifications (Rf ≥ 0.7).

For [^{68}Ga]Ga-PentixaFor solution, we performed the validation analysis on five synthesis, and all of them were in conformity with the specifications (mean Rf = 0.86 ± 0.01).

3.1.5 | pH test validation

The strip pH 0.0–6.0 humected with the pH 4.0 buffer has a color conform to a pH of 4 according to the color scale. pH strip moistened with DP solution has a pH of 5. The one moistened with pH 7.0 buffer is out of the scale. The strip pH 5.0–10.0 humected with the pH 7.0 buffer has a color conform to a pH of 7 according to the color scale. pH strip moistened with DP solution has a pH of 5. The one moistened with pH 4.0 buffer is out of the scale.

3.2 | Production and quality control of validation batches

The specifications and the quality controls of the validation batches are summarized in Table 2. For each batch, all the quality controls required by the European Pharmacopeia were done.

3.2.1 | Synthesis process

Validation batches were performed to validate the production process. The time of synthesis was between 16 and 18 min.

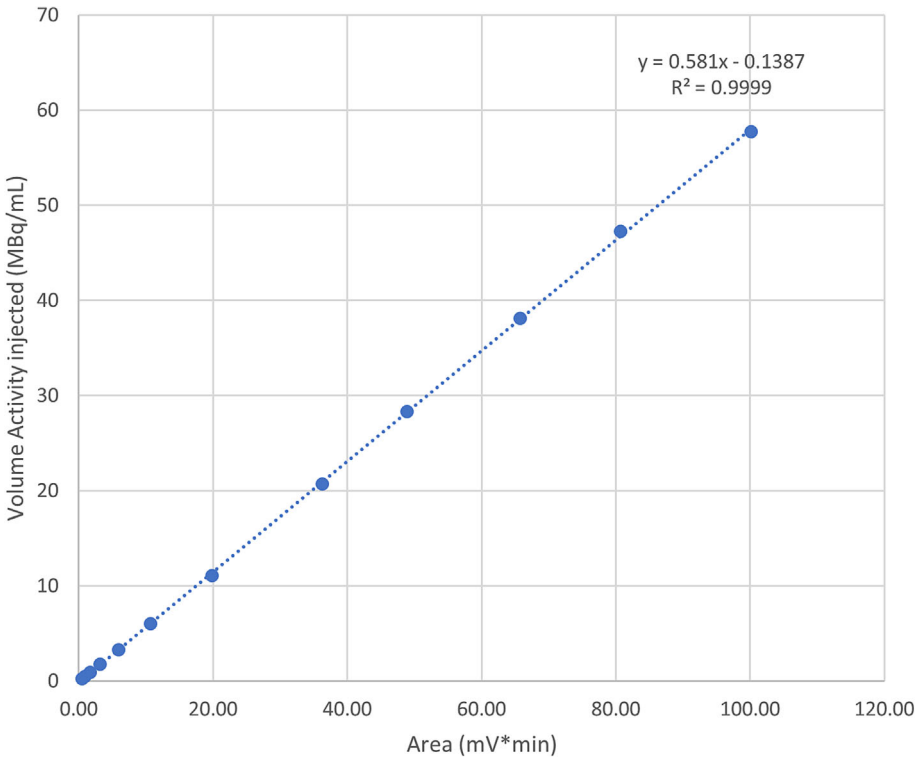

FIGURE 2 Concentration–peak area linear regression for RAD detector. The equation of the curve obtained is Y (activity per mL) = 0.581 × (peak area) − 0.1387.

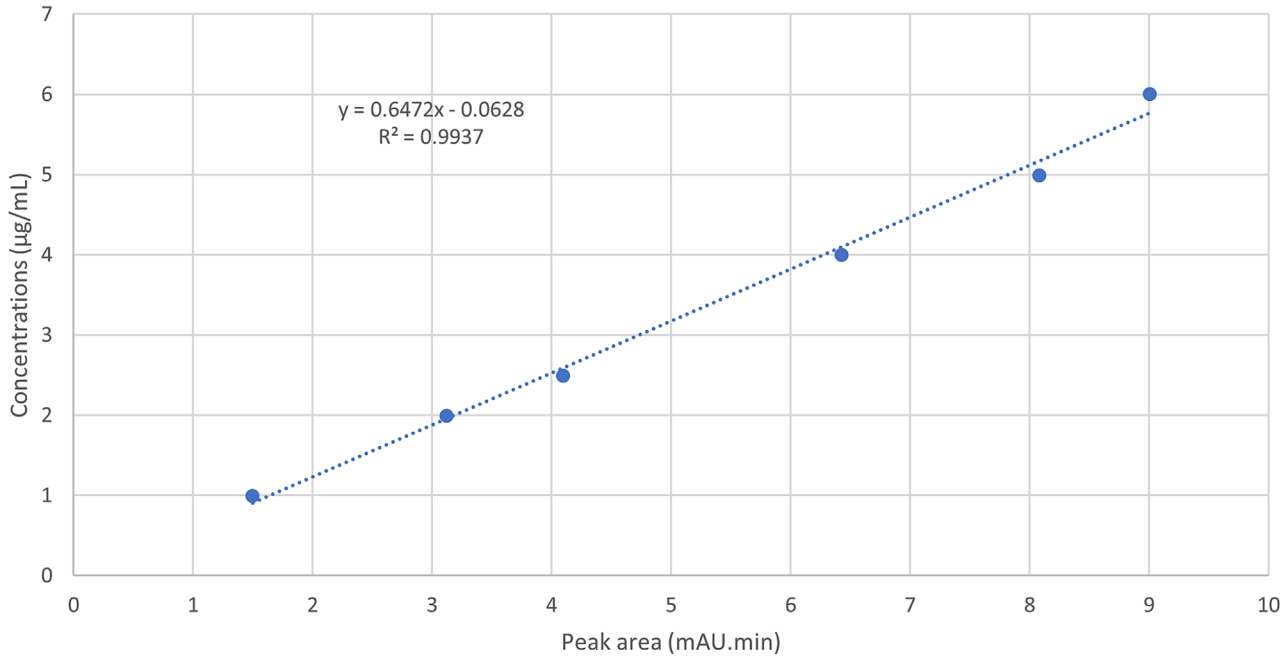

FIGURE 3 Concentration–peak area linear regression for UV detector. The equation of the curve obtained is Y (concentration) = 0.6472 × (peak area) − 0.0628.

TABLE 2 Summary of the specifications and results of the quality controls.

Test parameter	Method	Specification	Results
Appearance	Visual	Transparent and colorless solution.	Conform
pH	Strips	4.0–8.0	5.2 ± 0.26 ($n = 6$)
Filter integrity test	Bubble point test	≥2.5 bar	>3 bar ($n = 6$)
Radiochemical purity	RAD HPLC	[^{68}Ga]Ga-PentixaFor ≥ 95.0%	99.4 ± 0.4% ($n = 6$) (Lausanne) 99.9 ± 0.01% ($n = 3$) (Nantes)
	TLC	[^{68}Ga]Ga-PentixaFor ≥ 97.0%	99.5 ± 0.2% ($n = 5$) (Lausanne) 99.7 ± 0.03% ($n = 3$) (Nantes)
Peptide quantification	UV HPLC	Pentixafor ≤ 50 µg	4.0 ± 0.3 µg/mL ($n = 4$)
Ethanol content	GC	≤10%	8.5 ± 0.23% ($n = 4$) (Lausanne) 9.41 ± 0.07% ($n = 3$) (Nantes)
^{68}Ge breakthrough	Gamma counter	≤0.001%	0.00007% ($n = 6$)

Note: All the parameters were in conformity with the specifications of the International Conference Harmonization and the ^{68}Ga-DOTATOC monography in European Pharmacopeia.

The mean RCY estimated at the end of synthesis after decay correction was 94.8 ± 2.6% ($n = 6$) in Lausanne and 93.4 ± 0.7% ($n = 3$) in Nantes after decay correction. No significate difference was found between both sites ($p = 0.4$).

3.2.2 | Identification

On RAD HPLC, the mean retention time of [^{68}Ga]Ga-PentixaFor was 5.00 ± 0.07 min ($n = 4$). The relative retention time (RRT) between [^{68}Ga]Ga-PentixaFor and the reference compound [^{nat}Ga]Ga-Pentixafor was calculated to validate the identification. The RRT was 1.01 ± 0.01 ($n = 4$). The impurities identified were [^{68}Ga]$GaCl_3$ and two others unspecified impurities. Their retention times are shown in Table 3. An example of RAD HPLC chromatogram is provided in Figure 4A.

On TLC, the mean Rf obtained for [^{68}Ga] Ga-PentixaFor was 0.86 ± 0.01 ($n = 5$). The impurity found was unreacted ^{68}Ga on cationic form and colloidal form. The mean Rf obtained was 0.02 ± 0.01 ($n = 5$).

TABLE 3 Identification of the ^{68}Ga-impurities found in radio-HPLC by their mean retention times.

Impurity	Retention time
[^{68}Ga]$GaCl_3$	1.43 ± 0.01 min
Impurity 1	4.03 ± 0.07 min
Impurity 2	4.43 ± 0.06 min

Note: Results from Lausanne.

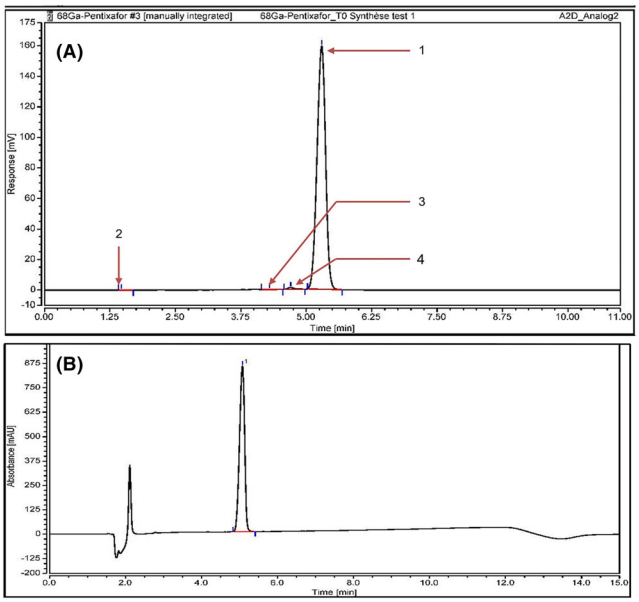

FIGURE 4 Example of HPLC chromatograms. (a) Radio-HPLC chromatogram of [^{68}Ga]Ga-PentixaFor at the end of synthesis with (1) [^{68}Ga]Ga-PentixaFor (Rt = 5.05 min), (2) Free [^{68}Ga]Ga (Rt = 1.44 min), (3) [^{68}Ga]Ga impurity 1 (Rt = 4.04 min), and (4) [^{68}Ga]Ga impurity 2 (Rt = 4.46 min). (b) UV chromatogram of [^{nat}Ga]Ga-Pentixafor.

3.2.3 | Radiochemical and ^{68}Ge breakthrough

The radiochemical purity (RCP) of the final drug product at end of synthesis (EOS) was evaluated by radio-HPLC and TLC. The RCP obtained is shown in Table 4. UV and RAD HPLC chromatograms of [^{68}Ga]Ga-PentixaFor and [^{nat}Ga]Ga-PentixaFor are represented on Figure 4. Radio-chromatogram obtained after TLC scan is presented in Figure 5.

The stability of the drug product was determined by measuring the RCP at 1 h, 2 h, and 3 h after the end of synthesis by radio-HPLC and TLC. The results obtained on both sites of production are presented in Table 5.

No significant differences of mean RCP at 1 h and 2 h were found between the two sites of production ($p > 0.05$).

TABLE 4 RCP obtained by HPLC and TLC on both sites of production.

Site of production	RCP by HPLC	RCP by TLC
Lausanne	99.4 ± 0.4% ($n = 6$)	99.5 ± 0.2% ($n = 5$)
Nantes	99.9 ± 0.01% ($n = 3$)	99.7 ± 0.03% ($n = 3$)

The ^{68}Ge breakthrough measured by decaying of an aliquot of eluate was $6.93.10^{-5} \pm 2.3.10^{-5}$% ($n = 6$).

3.2.4 | Peptide quantification

The amount of peptide injected to the patient was calculated with a maximum volume of injection (10 mL). In this condition, the amount of peptide injected would be 4.0 ± 0.3 µg/mL ($n = 4$), which is compliant with the specification.

3.2.5 | Residual solvent

The mean ethanol concentration in Lausanne and Nantes was, respectively, 8.5 ± 0.23% ($n = 4$) and 9.41 ± 0.07% ($n = 3$) They are lower than 10% in all the cases.

3.3 | Microbiology validation

The low bioburden of the cassette, peptide, and reagents was validated with 0 CFU/mL ($n = 3$).

All the validation batches ($n = 6$), the settle plates, and the agar for the gloves of the operator ($n = 6$) were sterile.

The endotoxins content of the drug product was <2.00 EU/mL ($n = 3$).

4 | DISCUSSION

In this work, we established successfully the production of [^{68}Ga]Ga-PentixaFor with the Trasis miniAiO module, the quality control of the drug product, and the validation of the analytical procedure. This synthesis was already described with other synthesizers like Scintomics,[14,15] Modular Lab Pharm Tracer (Eckert & Ziegler),[16] or EAZY® (Eckert & Ziegler)[19] but not on miniAiO so far.

For the production process, several developments were performed. The heating conditions were set up regarding those established in previous reports. Optimal

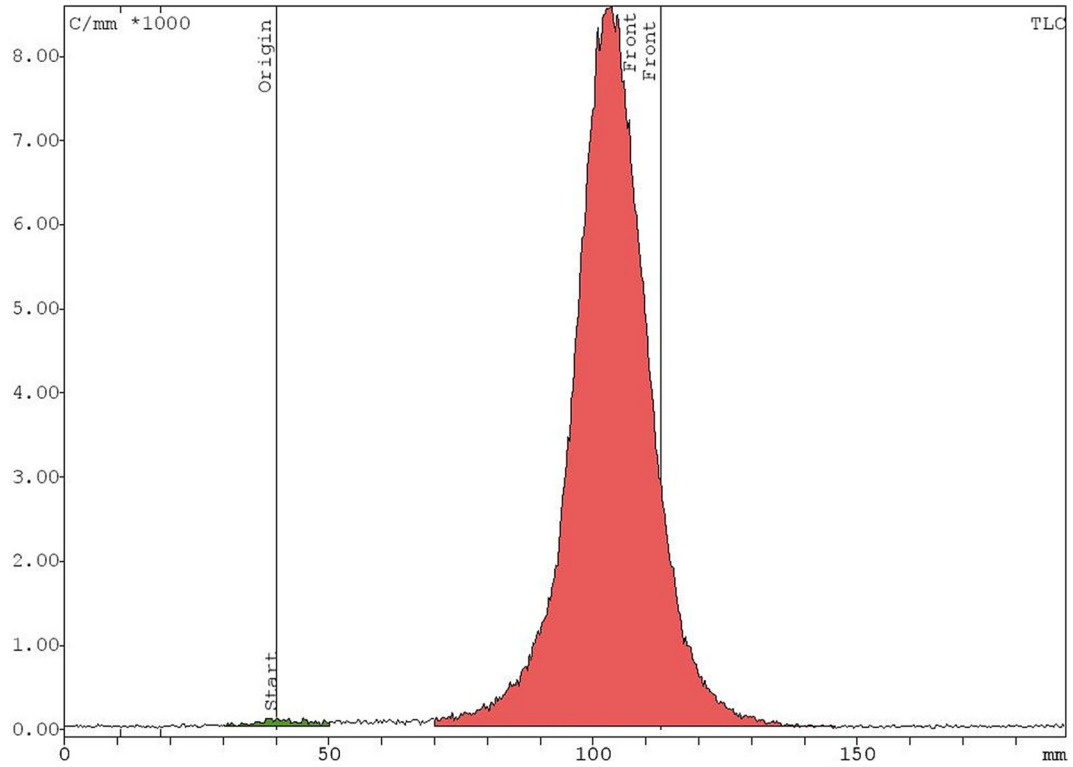

FIGURE 5 Example of TLC chromatogram of [^{68}Ga]Ga-PentixaFor at the end of synthesis. Rf obtained for [^{68}Ga]Ga-PentixaFor and impurities were, respectively, 0.86 ± 0.01 and 0.02 ± 0.01.

TABLE 5 Radiochemical purity on the [^{68}Ga]Ga-PentixaFor over time.

Site of production	Time of measuring	RCP by HPLC	RCP by TLC
Lausanne	1 h	99.0 ± 1.3%	99.7 ± 0.2%
	2 h	99.1 ± 1.1%	99.6 ± 0.1%
	3 h	99.5 ± 0.4%	99.5 ± 0.4%
Nantes	1 h	99.7 ± 0.3%	99.7 ± 0.03%
	2 h	99.7 ± 0.05%	99.9 ± 0.1%

Note: The RCP was determined by HPLC and TLC ($n = 3$) at 1 h, 2 h, and 3 h after the end of synthesis to evaluate the stability of the drug product.

conversion temperature of 95°C was selected resulting in an acceptable time and radio chemical yield. However, to avoid the heating of the peptide in the buffer alone, no pre-heating step was performed. The temperature of the reaction vial was set first at 120°C during the first 90 s to reach 95°C inside the reactor faster, and then lowered to 95°C, avoiding a too long heating of the peptide, which could degrade it. The duration of the labelling process (16–18 min) remains close to other peptides radiolabelled with the MiniAiO modules.[20] With these parameters, we obtained [^{68}Ga]Ga-PentixaFor with a high RCY (94.8 ± 2.6%, decay corrected) and a high RCP on HPLC (99.4 ± 0.4%) and TLC (99.5 ± 0.5%). Furthermore, the RCY and RCP obtained at EoS, 1 h and 2 h in Nantes and Lausanne, are very similar. These results showed the robustness of the automated production in these conditions.

Various impurities generated from the column (Ti^{4+}), the decay of ^{68}Ga (Zn^{2+}), or other sources of metallic contamination (Fe^{3+}) can disturb the labelling reaction and decrease the RCP.[21] The radiolabeling yield depends on several parameters as the metal/ligand ratio, the pH, or the temperature.[22] Contrary to other groups that described a prepurification step,[16,20] we chose to develop a process without prepurification of the ^{68}Ge/^{68}Ga generator eluate. We did not observe any impact on the quality of the final product due to the very low level of impurities in the eluate of new generators with European marketing authorization (e.g., GalliaPharm). Furthermore, we found a mean ^{68}Ge amount ($6.93.10^{-5} \pm 2.3.10^{-5}$%) hundred times below the specifications provided by the supplier ($<10^{-3}$%).

The final product was validated with a stability of 3 h after synthesis (or delivery in the final product vial). We

did not evaluate the stability over a longer period regarding the short half-life of ^{68}Ga. The RCYs are comparable to those obtained in previous studies done with other synthesizers.[16]

The amount of peptide available for the radiolabeling influences the yield of production and the activity in the drug product at the end of synthesis. Indeed, Spreckelmeyer et al. demonstrated that the RCYs obtained immediately after synthesis ($n \geq 3$) with 20, 30, and 50 μg of peptide were, respectively, 18.9 ± 2.4%, 71.0 ± 10.6%, and 80.9 ± 10.0% after decay correction.[16] Furthermore, Nader et al. obtained also a better RCY with 50 μg of peptide (95%) compared to 10 μg (60%) and 30 μg (89%).[19] Starting precursor amounts lower than 50 μg provide insufficient conversion rate and hence reduced radiolabeling yields. Moreover, following the microdose concept, an extended single dose toxicity study in New Zealand white rabbits was performed, justifying injected peptide dose of up to 100 μg per patient (unpublished data from PentixaPharm GmbH). Hence, the use of 50 μg of net peptide for the radiolabeling is considered safe. Thus, a starting amount of 50 μg of PentixaFor precursor has been deemed as suitable. On our validation batches in Lausanne and Nantes, the radiochemical yields obtained with 50 μg of Pentixafor were, respectively, 94.8 ± 2.6% and 93.4 ± 0.7% confirming the previous results. The differences of RCY observed between Modular Lab and miniAiO synthesizers can be explained by the duration of the radiolabeling. Indeed, the reaction was performed during 5 min with the Modular Lab PharmTracer versus 10 min with the miniAiO at the same temperatures. The RCY obtained with the Eazy modular synthesis is consistent with this hypothesis because the temperature and the duration of incubation are longer than Spreckelmeyer and al.

[^{68}Ga]Ga-PentixaFor has no monography described in the Eur. Ph. Thus, we performed a validation of the analytical method according to the ICH recommendations (CPMP/ICH/381/95).[23,24] The specifications of the quality control were defined according to the Eur. Ph. of the [^{68}Ga]Ga-edotreotide, which has the same DOTA chelator.[25] All the parameters were complied with the specifications defined in the [^{68}Ga]Ga-edotreotide monography. We validated a HPLC method that allows to identify the [^{68}Ga]Ga-PentixaFor and to quantify it in the final product.

For the release of the Drug Product, the HPLC method developed and described in this work is able to separate [^{68}Ga]Ga-PentixaFor from [^{68}Ga]Ga-L-Orn-PTF, an impurity surrogate for likely peptide-related impurities even in the hypothetical case where both peptides would be present in a 1:1 ratio.

For the SST, [^{nat}Ga]Ga-Pentixafor was analyzed using our HPLC method under concentrations similar to those to be expected in the Drug Product (5 μg/mL). The RRT between [^{nat}Ga]Ga-Pentixafor and [^{68}Ga]Ga-PentixaFor was very close to 1.0 (RRT = 0.995; CV = 0.2%) showing that this compound can be used as reference standard for the radioactive form. In these conditions, we obtained signals with good quality and reproducibility (symmetry factor with CV = 0.6%). The parameters of identification (retention time) and quantification (peak area) showed a great reproducibility as well with CV, respectively, of 0.1% and 0.6%.

The TLC method allows to separate the [^{68}Ga]Ga-PentixaFor from the impurities corresponding to unreacted ^{68}Ga on colloidal and cationic forms.

The mean residual ethanol measured by gas chromatography in Lausanne and Nantes was, respectively, 8.5 ± 0.2% and 9.41 ± 0.07%, whereas the specification is <10%. All the batches complied with the specifications. We compared these results to those obtained with the same cassette, the same reagents kits, and the same volume of ethanol on the MiniAiO but for two other synthesis processes performed in our radiopharmacy unit, namely, [^{68}Ga]Ga-PSMA-11 ($n = 273$) and [^{68}Ga]Ga-DOTATOC synthesis ($n = 292$). The mean residual ethanol obtained for these productions was 7.4 ± 1.5% showing the strong reproducibility of the elution step by ethanol and the relevance to do this quality control after the injection to the patient for radioprotection purposes.

For microbiological analysis, the low bioburden, the sterility or the absence of endotoxin contamination were approved on the validation batches. No contamination of the environment was found for all batches. All these results conjugated with the use of class A hot cell confirmed the safety of this automated aseptic process.

5 | CONCLUSION

In this work, we successfully developed the automated production of [^{68}Ga]Ga-PentixaFor on miniAiO synthesizer and validated the quality control of this radiopharmaceutical.

The reproducibility, the cost and the availability of this in house production allows to increase the access of the patient to this new promising radiopharmaceutical.

ACKNOWLEDGMENTS

We are grateful to PentixaPharm for providing the PentixaFor precursor used in this work. We thank the staff members of the radiopharmacy units from Lausanne and Nantes involved in the production and analytical process. Open access funding provided by Universite de Lausanne.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

ETHICS STATEMENT

Not applicable.

ORCID

Julien Costes https://orcid.org/0000-0002-4528-7362
Judith Anna Delage https://orcid.org/0000-0003-3039-5173

REFERENCES

1. Lira SA, Furtado GC. The biology of chemokines and their receptors. *Immunol Res*. 2012;54(1-3):111-120. doi:10.1007/s12026-012-8313-7
2. Britton C, Poznansky MC, Reeves P. Polyfunctionality of the CXCR4/CXCL12 axis in health and disease: implications for therapeutic interventions in cancer and immune-mediated diseases. *FASEB j*. 2021;35(4):e21260. doi:10.1096/fj.202001273R
3. Pozzobon T, Goldoni G, Viola A, Molon B. CXCR4 signaling in health and disease. *Immunol Lett*. 2016;177:6-15. doi:10.1016/j.imlet.2016.06.006
4. Burger JA, Kipps TJ. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. *Blood*. 2006;107(5):1761-1767. doi:10.1182/blood-2005-08-3182
5. Kircher M, Herhaus P, Schottelius M, et al. CXCR4-directed theranostics in oncology and inflammation. *Ann Nucl Med*. 2018;32(8):503-511. doi:10.1007/s12149-018-1290-8
6. Döring Y, Pawig L, Weber C, Noels H. The CXCL12/CXCR4 chemokine ligand/receptor axis in cardiovascular disease. *Front Physiol*. 2014;5:212.
7. Karin N. The multiple faces of CXCL12 (SDF-1α) in the regulation of immunity during health and disease. *J Leukoc Biol*. 2010;88(3):463-473. doi:10.1189/jlb.0909602
8. Woodard LE, Nimmagadda S. CXCR4-based imaging agents. *J Nucl Med off Publ Soc Nucl Med*. 2011;52(11):1665-1669.
9. Tsutsumi H, Tanaka T, Ohashi N, et al. Therapeutic potential of the chemokine receptor CXCR4 antagonists as multifunctional agents. *Pept Sci*. 2007;88(2):279-289. doi:10.1002/bip.20653
10. Demmer O, Dijkgraaf I, Schumacher U, et al. Design, synthesis, and functionalization of dimeric peptides targeting chemokine receptor CXCR4. *J Med Chem*. 2011;54(21):7648-7662. doi:10.1021/jm2009716
11. Luker GD, Yang J, Richmond A, et al. At the bench: preclinical evidence for multiple functions of CXCR4 in cancer. *J Leukoc Biol*. 2021;109(5):969-989. doi:10.1002/JLB.2BT1018-715RR
12. Buck AK, Stolzenburg A, Hänscheid H, et al. Chemokine receptor—directed imaging and therapy. *Methods San Diego Calif*. 2017;130:63-71. doi:10.1016/j.ymeth.2017.09.002
13. Herrmann K, Lapa C, Wester HJ, et al. Biodistribution and radiation dosimetry for the chemokine receptor CXCR4-targeting probe ^{68}Ga-Pentixafor. *J Nucl Med*. 2015;56(3):410-416. doi:10.2967/jnumed.114.151647
14. Sammartano A, Migliari S, Scarlattei M, Baldari G, Ruffini L. Synthesis, validation and quality controls of [^{68}Ga]-DOTA-pentixafor for PET imaging of chemokine receptor CXCR4 expression. *Acta Bio Medica Atenei Parm*. 2020;91(4):e2020097.
15. Watts A, Chutani S, Arora D, et al. Automated radiosynthesis, quality control, and biodistribution of Ga-68 Pentixafor: first Indian experience. *Indian J Nucl Med IJNM off J Soc Nucl Med India*. 2021;36(3):237-244.
16. Spreckelmeyer S, Schulze O, Brenner W. Fully-automated production of [^{68}Ga]Ga-PentixaFor on the module Modular Lab-PharmTracer. *EJNMMI Radiopharm Chem*. 2020;5(1):8. doi:10.1186/s41181-020-0091-2
17. Elsinga P, Todde S, Penuelas I, et al. Guidance on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals. *Eur J Nucl Med Mol Imaging*. 2010;37(5):1049-1062. doi:10.1007/s00259-010-1407-3
18. Gillings N, Hjelstuen O, Ballinger J, et al. Guideline on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals. *EJNMMI Radiopharm Chem*. 2021;6(1):8. doi:10.1186/s41181-021-00123-2
19. Nader M, Herrmann K, Kunkel F, et al. Improved production of ^{68}Ga-Pentixafor using cartridge mediated cation exchange purification. *Appl Radiat Isot*. 2022;189:110447. doi:10.1016/j.apradiso.2022.110447
20. Sørensen MA, Andersen VL, Hendel HW, et al. Automated synthesis of ^{68}Ga/^{177}Lu-PSMA on the Trasis miniAllinOne. *J Label Compd Radiopharm*. 2020;63(8):393-403. doi:10.1002/jlcr.3846
21. Boschi S, Lodi F, Malizia C, Cicoria G, Marengo M. Automation synthesis modules review. *Appl Radiat Isot*. 2013;76:38-45. doi:10.1016/j.apradiso.2012.09.010
22. Oehlke E, Le VS, Lengkeek N, et al. Influence of metal ions on the ^{68}Ga-labeling of DOTATATE. *Appl Radiat Isot Data Instrum Methods Use Agric Ind Med*. 2013;82:232-238. doi:10.1016/j.apradiso.2013.08.010
23. Gillings N, Todde S, Behe M, et al. EANM guideline on the validation of analytical methods for radiopharmaceuticals. *EJNMMI Radiopharm Chem*. 2020;5(1):7. doi:10.1186/s41181-019-0086-z
24. Todde S, Peitl PK, Elsinga P, et al. Guidance on validation and qualification of processes and operations involving radiopharmaceuticals. *EJNMMI Radiopharm Chem*. 2017;2(1):8. doi:10.1186/s41181-017-0025-9
25. Decristoforo C, Pickett RD, Verbruggen A. Feasibility and availability of ^{68}Ga-labelled peptides. *Eur J Nucl Med Mol Imaging*. 2012;39(1):31-40. doi:10.1007/s00259-011-1988-5

How to cite this article: Costes J, Casasagrande K, Dubegny C, et al. [^{68}Ga]Ga-PentixaFor: Development of a fully automated in hospital production on the Trasis miniAllinOne synthesizer. *J Label Compd Radiopharm*. 2023;66(12):400-410. doi:10.1002/jlcr.4061