

METHODOLOGY

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Fully-automated production of [⁶⁸Ga]Ga-PentixaFor on the module Modular Lab-PharmTracer

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Abstract

Background: PentixaFor is a promising radiopharmaceutical for positron emission tomography in the detection of different tumor entities and other diseases. Until now, the synthesis of [⁶⁸Ga]Ga-PentixaFor was reported for the automated synthesis module from Scintomics® only. Our aim was to evaluate the automated synthesis of this radiopharmaceutical on a different module in order to make it available for a broader community.

Results: The synthesis of [⁶⁸Ga]Ga-PentixaFor with different amounts of PentixaFor (50 µg, 30 µg and 20 µg) on the Modular Lab PharmTracer (MLPT) from Eckert & Ziegler with the already established synthesis template for [⁶⁸Ga]Ga-DOTATOC yielded best results with 50 µg PentixaFor for clinical multi-dose application. All different quality control parameters tested (e.g. sterility, stability and radiochemical purity) were in accordance with the European Pharmacopoeia.

Conclusions: [⁶⁸Ga]Ga-PentixaFor was successfully synthesized fully-automated on the synthesis module Modular Lab PharmTracer and can be used for multi-dose application in clinical settings.

Keywords: Automated production, Imaging, CXCR4, PentixaFor

Background

In 2011, Demmer et al. first introduced chemokine receptor-4 (CXCR4)-binding peptidic probes for molecular imaging (Demmer et al., 2011). PentixaFor (Synonym for CPC4.2, Fig. 1) is to date the most promising peptide with a high affinity to chemokine receptor-4. PentixaFor can be used as PET imaging agent when coupled to the positron emitting radionuclide gallium-68. Numerous clinical studies have already been performed in vivo and the interest is steadily increasing in new applications for different tumor entities and other diseases, e.g. inflammatory conditions (Bouter et al., 2018). Examples of clinical applications in oncology are the detection of neuroendocrine tumors, multiple myeloma (Pan et al., 2018), glioma, leukemia (Mayerhoefer et al., 2018) or lymphoma (Luo et al., 2019; Haug et al., 2019). Since the precursor PentixaFor was made available until summer 2018 only for use by the synthesis module from Scintomics®, only hospitals that operated a Scintomics® module were able to prepare [⁶⁸Ga]Ga-PentixaFor. Here, we would like to introduce the synthesis conditions of [⁶⁸Ga]Ga-PentixaFor on a different synthesis module, namely Modular Lab PharmTracer (MLPT) from Eckert & Ziegler Eurotope

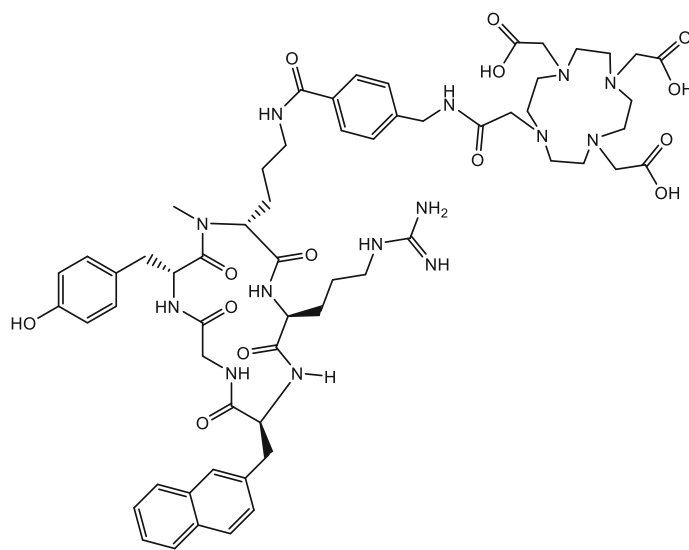


Fig. 1 Structure of PentixaFor (molecular weight: 1221.4 g/mol)

GmbH – in order to introduce [^{68}Ga]Ga-PentixaFor to a broader community. For that purpose, different concentrations of PentixaFor were evaluated based on quality control parameters. Concerning the synthesis of ^{68}Ga -tracers in general, different modules are available on the market which have been reviewed by Boschi et al. In addition, generator post-processing steps like fractionation, anionic-exchange and cation-exchange are described (Boschi et al., 2013). The now proposed fully automated pre-purification of gallium-68 on a strong cation exchange (SCX) cartridge has the advantage that gallium-68 becomes concentrated and germanium-68 as well as non-radioactive impurities (e.g. Zn^{2+}) are trapped on the SCX cartridge.

Results

Labeling results of different amounts of PentixaFor

The fully automated production of [^{68}Ga]Ga-PentixaFor was conducted on the commercial labeling synthesis module MLPT. Three different precursor amounts of PentixaFor were tested for radiolabeling with 1080–2500 MBq of gallium-68 (three generators from type GalliaPharm®) - 50 μg , 30 μg and 20 μg . As shown in Table 1, the highest amount of PentixaFor (50 μg) resulted in 94.7% radiochemical yield (RCY, n.d.c.) in the product vial, 2.6% in the waste vial and 1.7% on the C_{18} cartridge. By lowering the amount of PentixaFor to 30 μg , the RCY of the product decreased to 80.5% (n.d.c.) and the amount on the C_{18} cartridge increased to 13.3% (n.d.c.). By lowering the amount of starting material to 20 μg , the efficiency became worse. Only 20.0% (n.d.c.) of radiochemical yield was found

Table 1 Activity measured on different parts of the cassette immediately after synthesis ($n \geq 3$)

	Product n.d.c. [%]	Waste n.d.c. [%]	C_{18} cartridge n.d.c. [%]	RCY (decay corrected) [%]
50 μg	94.7 \pm 0.7	2.6 \pm 0.5	1.7 \pm 0.5	80.9 \pm 10.0
30 μg	80.5 \pm 4.2	2.9 \pm 1.8	13.3 \pm 4.5	71.0 \pm 10.6
20 μg	20.0 \pm 3.2	9.3 \pm 1.2	68.4 \pm 2.5	18.9 \pm 2.4

in the product vial and 69.4% on the C₁₈ cartridge. The distribution of the radioactivity in the cassette for the productions is presented in Fig. 2.

The radioactivity found in the reactor and SCX cartridge did not change significantly by changing the amount of starting material.

In the waste fraction, with decreasing peptide amounts from 50 to 20 µg, the radioactivity increased from 2.6% to 9.3%. Interestingly, the activity measured in the waste vial consists of approximately 25% product and 75% free gallium-68 as seen in the radio-HPLC chromatogram as well as in the radio-iTLC (Supporting Information, Fig. 1).

Quality control of [⁶⁸Ga]Ga-PentixaFor

The radiochemical purity was evaluated with a standard protocol by radio-HPLC and radio-iTLC.

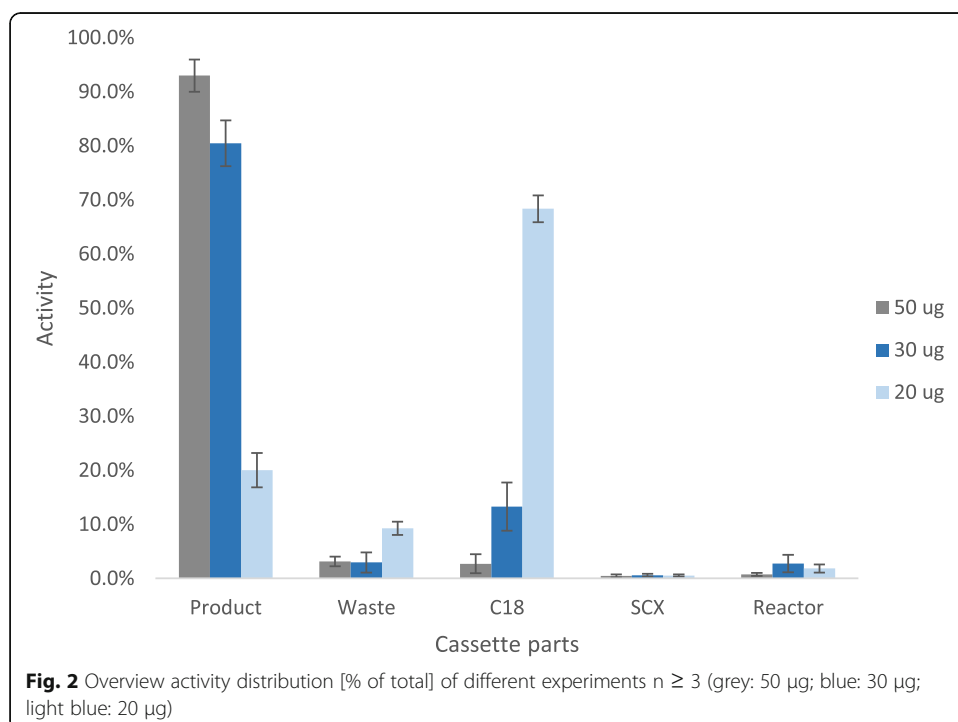
With radio-HPLC, free gallium-68 would be detected at $t_R = 2.5$ min, whereas gallium-68 bound to PentixaFor was detected at $t_R = 11$ min. Radioactive impurities could not be detected with this method (Fig. 3a).

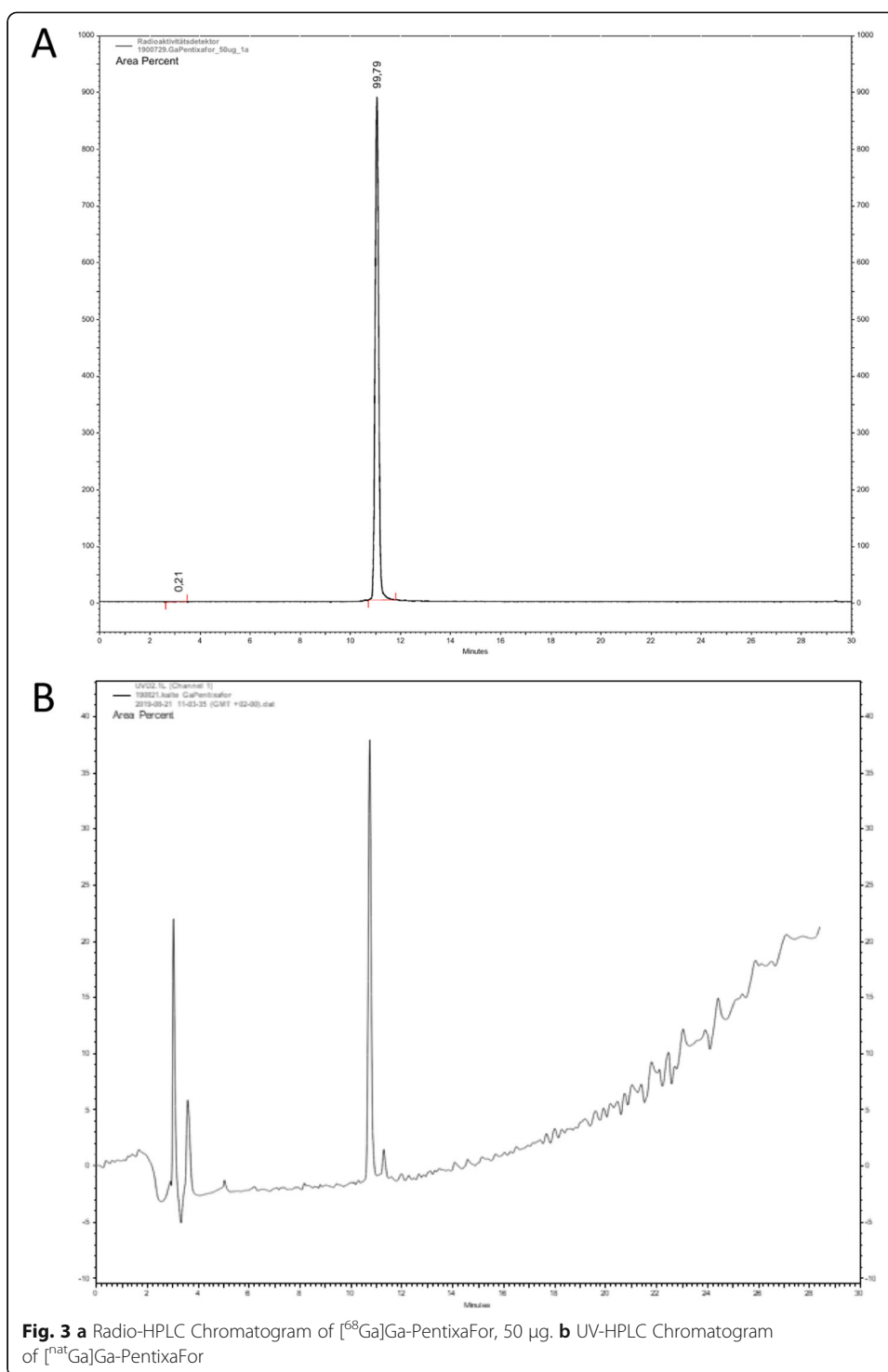
With radio-iTLC, no ⁶⁸Ga-colloide could be detected at $R_f = 0.2$ but the product at $R_f = 0.8$ (Fig. 4).

The cold standard [^{nat}Ga]Ga-PentixaFor shows a similar retention time of $t_R = 11$ min as seen in Fig. 3b.

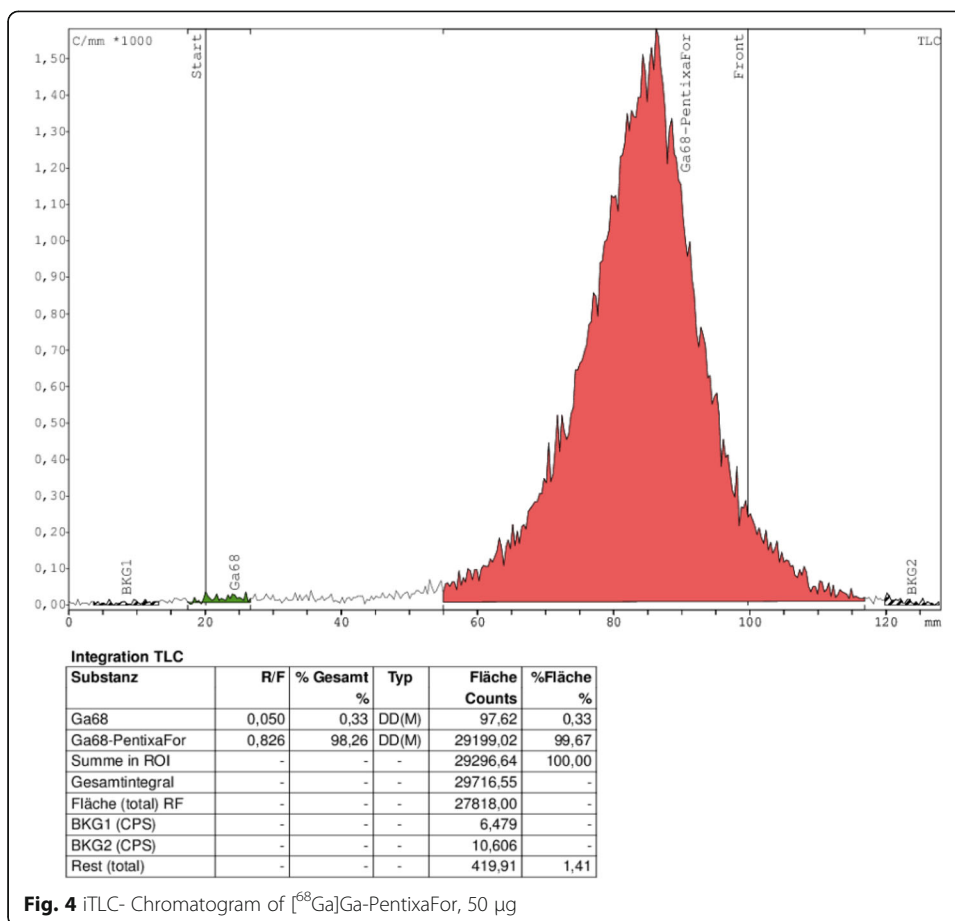
In addition, the product solution was tested for endotoxins. For this purpose, the solution was diluted with endotoxin-free-water in a ratio 1:10. For all samples, an endotoxin concentration below 0.5 IE/mL was detected. This is in accordance with the European Pharmacopoeia (9.0/0125).

With regards to sterility, all products were sterile.





The stability of $[^{68}\text{Ga}]\text{Ga-PentixaFor}$ in aqueous solution at room temperature was tested up to 4 h via radio-HPLC. As seen in Fig. 5, $[^{68}\text{Ga}]\text{Ga-PentixaFor}$ is stable at those conditions. No radioactive by-products or free gallium-68 could be detected during this time period.

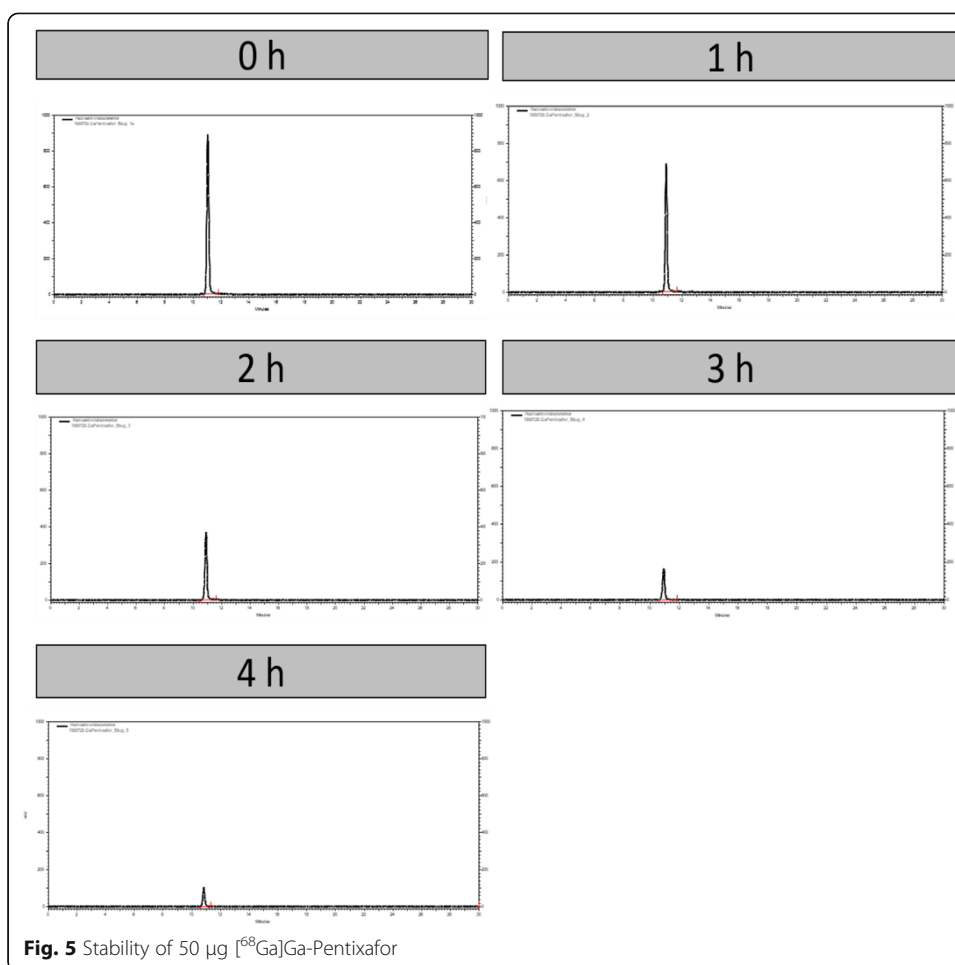


In Table 2, product specifications of the most promising approach with 50 µg PentixaFor are summarized. Acceptance criteria are based on the European Pharmacopoeia (9.0/0125).

Discussion

For establishing the automated synthesis of [⁶⁸Ga]Ga-PentixaFor on the Modular Lab PharmTracer from Eckert & Ziegler Eurotope GmbH for the first time, three different amounts of PentixaFor were evaluated for the production of this tracer with the same standard synthesis template as used for [⁶⁸Ga]Ga-DOTATOC on the synthesis module MLPT. The results summarized in Table 1 and Fig. 2 demonstrate that the amount of 50 µg of PentixaFor yields the best radioactivity distribution on the cassette. The low standard deviations for 50 µg confirm the high reproducibility of the production.

For 30 µg and 20 µg, an increasing amount of radioactivity on the C₁₈ cartridge and a decreasing yield of activity in the product vial was observed. Theoretically, two scenarios are to our knowledge possible to explain the increased radiochemical yield on the C₁₈ cartridge. Either the product is trapped on the C₁₈ cartridge or uncomplexed ⁶⁸Ga-colloids. Product could be eluted from the C₁₈ cartridge with pure ethanol. After the automated synthesis is completed, we tried to elute the activity. This resulted in no change of activity measured on the C₁₈ cartridge. As a consequence, no product is stuck on the C₁₈ cartridge. The most likely explanation is that 20 µg and 30 µg are too



low to complex $^{68}\text{Ga}^{3+}$ ions completely resulting in a trapping of unbound gallium-68 in the form of $^{68}\text{Ga}^{3+}$ -colloids on the C_{18} cartridge.

Based on the analysis of the waste fraction by radio-HPLC and radio-iTLC, 25% of the activity found in the waste fraction was product. With respect to the total radioactivity used in 50 µg experiments, this means a negligibly product activity loss of under 1%.

The minimum amount needed to fully complex PentixaFor under the described conditions was 50 µg. In former studies in which ^{68}Ga]Ga-PentixaFor was synthesized on a synthesis module from Scintomics[®], 40 µg and 20 µg of PentixaFor were used in a final product volume of 15 mL and 14 mL, respectively. The amount of PentixaFor that is safe to be used in vivo is therefore < 20 µg per patient based on those results (Lapa et al., 2016; Wester et al., 2015). In the frame of the here described synthesis protocol, it is important to make sure via standard operating procedures (SOPs), that the maximal injected amount of tracer is below 20 µg/patient. Thus, the maximum injected volume needs to be below 3.6 mL.

With regard to the quality control parameters, we achieved an endotoxine-free, sterile and stable solution of ^{68}Ga]Ga-PentixaFor with a radiochemical purity > 95% over a period of 4 h. Moreover, all tested quality control parameters were in accordance with the European Pharmacopoeia (see Table 2). Calculating the ethanol content based on

Table 2 Summary of the product specifications for 50 µg PentixaFor, n ≥ 3

Test	Acceptance criteria	[⁶⁸ Ga]Ga-PentixaFor
Radiochemical purity (radio-HPLC)	> 95%	99.8%
Radiochemical purity (radio-iTLC)	> 95%	99.7%
pH	4–8.5	5
Radioactivity concentration	> 50 MBq/ mL	120–190 MBq/ mL
Radioactivity	> 150 MBq	1080–1700 MBq (depending on amount of generators used)
Volume	2–10 mL	9 mL
Color	Colorless	Colorless
Molar radioactivity	1–60 MBq/ nmol	27–42.5 MBq/nmol
Radionuclidic purity	> 99.9%	99.9 %
⁶⁸ Ge breakthrough	< 0.001%	0.0002%
Endotoxins	< 19.0 IE/mL	< 0.5 IE/mL
Stability over 4 h	> 90%	99.9%
EtOH content	< 10%	≤ 6.2% (calculated)

the parameters chosen in the synthesis template of the MPLT software, results in an ethanol content that cannot exceed 6.2%. Nevertheless, we recommend to either measure the ethanol content of every batch by gas chromatography before releasing it for clinical application, or validate the ethanol content by an external laboratory if a gas chromatography is not available onsite.

Conclusions

[⁶⁸Ga]Ga-PentixaFor was successfully synthesized fully-automated on the synthesis module MLPT for the first time. All the tested quality parameters for the radiochemical purity, pH, endotoxins and sterility are in accordance with the European Pharmacopoeia. In addition, the product solution is stable for at least 4 h after production, as shown by radio-HPLC. Thus, [⁶⁸Ga]Ga-PentixaFor can be easily and reliably produced on the module MLPT for clinical application.

Methods

Materials

PentixaFor was obtained from PentixaPharm. An aqueous stock solution of 1 mg/mL was prepared and kept at –15 °C. All chemicals were of pure chemical grade and solvents for high-pressure-liquid-chromatography (HPLC) were obtained as HPLC grade. TraceSelect water (Sigma-Aldrich) was used in all experiments. The pharmaceutical grade ⁶⁸Ge/⁶⁸Ga generator (GalliaPharm®, Eckert & Ziegler Radio-pharma GmbH, Germany), Modular Lab PharmTracer (Eckert & Ziegler Eurotope GmbH, Germany) and reagent set EZ-102 (Eckert & Ziegler Eurotope GmbH, Germany) were used. The amount of detected metal impurities/ ⁶⁸Ge breakthrough as provided by the manufacturer was less than the defined limit in the European Pharmacopoeia monograph. Activity counting was determined using a borehole counter (Nuklear-Medizintechnik Dresden GmbH, Germany). HPLC was performed using the HPLC system Knauer Azura (UVD: 2.1 L; P6.1L) coupled with UV and

radiometric (Raytest Socket 2"8103 0370) detectors. The TLC scanner used was MiniGita from Raytest. The test for endotoxins was performed using Nexgen PTS (Charles River).

Preparation for labeling of PentixaFor with gallium-68

First, the commercial fully automated synthesis platform MLPT was equipped with a disposable single-use cassette (C4-GA-PEP). The synthesis template and buffer preparations were identical to the ones used for [^{68}Ga]Ga-DOTATOC. All synthesis reagents were contained in the reagent set except the peptide. From the reagent set, 50 mL of 0.9% NaCl and 10 mL of EtOH/H₂O (50/50) solution were connected to the cassette on the designated spikes. After connection of the waste vial the preconditioning of the C₁₈ cartridge was performed automatically without user interaction. During the conditioning step PentixaFor was prepared in 20 µg, 30 µg and 50 µg aliquots from the stock solution (1 mg / mL). Meanwhile, the preparation of the acetate buffer solution was performed according to the user manual using EZ 102. The reaction mixture contains 2.2 mL of the final buffer solution, 0.4 mL of EtOH/H₂O (50/50) and an aliquot of PentixaFor, which was loaded into the reaction vial. The required volume of eluent was added to the eluent vial prior to synthesis.

Labeling of PentixaFor with $^{68}\text{GaCl}_3$

The synthesis was performed fully-automated without any user interaction. $^{68}\text{Ga}^{3+}$ obtained from a 1.850 MBq $^{68}\text{Ge}/^{68}\text{Ga}$ generator (GalliaPharm®) with TiO₂ matrix, was eluted with 0.1 N HCl. The generator eluate was pre-concentrated on a strong cation exchange (SCX) cartridge. [$^{68}\text{GaCl}_4$]⁻ was recovered from the SCX cartridge by the eluent (5 M NaCl/HCl (0.1 M)) (Schultz et al., 2013). The reaction vial containing the reaction mixture was preheated to 50 °C, and after the ^{68}Ga -activity was transferred to the reaction vial, the temperature was increased to 95 °C. After 5 min at 95 °C, the reaction mixture was cooled down by adding 3 mL of 0.9% NaCl. The crude reaction solution was subsequently transferred to the C₁₈ cartridge for purification. The final product was eluted from the C₁₈ cartridge using a EtOH/H₂O (50/50) solution and passed through a 0.22 µm sterile filter into the sterile product vial. The resulting product was diluted to 9 mL with 0.9% NaCl. A sample for quality control was taken.

Quality control

After synthesis, the product vial is removed from the MLPT module and further evaluated for quality control determining the following parameters: total product activity, $^{68}\text{Ga}^{3+}$ -identity via half-life time, chemical purity (pH, sterility and endotoxins) and radiochemical purity (high-pressure-liquid chromatography). The stability of the product at room temperature was monitored by radio-HPLC for 4 h. Radio-HPLC was performed with a standardized method. A: water + 0.1% TFA; B: acetonitrile + 0.1% TFA, gradient: 0–25 min A: 0–100%; 25–28 min A: 100–0%. The limit of detection of the radio-HPLC is 10 kBq/ 20 µL injected volume and the recovery is approximately 80%. For iTLC, ammonium acetate/methanol (1/1) was used as mobile phase and iTLC-SG strips as stationary phase. The sterility tests were performed as described in the European Pharmacopoeia at the Institut für Hygiene, Charité.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s41181-020-0091-2>.

Additional file 1. Supporting Information, Fig. 1: iTLC chromatogram of the waste fraction

Abbreviations

$^{68}\text{Ga}^{3+}$: ^{68}Ga Gallium³⁺ ion; $^{68}\text{Ge}^{4+}$: ^{68}Ge Germanium⁴⁺ ion; CXCR4: Chemokine receptor-4; HPLC: High-pressure-liquid-chromatography; MLPT: Modular Lab PharmTracer; PET: Positron Emission Tomography; RCY: Radiochemical yield; TFA: Trifluoro acetic acid

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Authors' contributions

SS designed, performed and analyzed the experiments. OS designed the evaluation of quality control parameters and repeated the experiments in order to proof its repeatability. WB evaluated the results concerning clinical applications. All authors contributed to, read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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