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Posted Date: 14 September 2023

doi: 10.20944/preprints202309.0931.v1

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Article

Fully Automated Production of [^{68}Ga]FAPI-46 with Gallium-68 from Cyclotron Using Liquid Targets

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Abstract: ^{68}Ga-based radiopharmaceuticals are routinely used for PET imaging of multiple types of tumors tumours. Gallium-68 is commonly obtained from ^{68}Ge/^{68}Ga generators, which are limited in the quantity of activity produced. Alternatively, gallium-68 can easily be produced on a cyclotron using liquid targets. In this study we optimized the GMP production of [^{68}Ga]GaFAPI-46 using gallium-68 produced from a standard medical cyclotron using liquid targets. Starting from the published synthesis and quality control procedures described for other ^{68}Ga-based radiopharmaceuticals, we have validated the synthesis process and the analytical methods to test the quality parameters of the final product to be used for routine clinical studies. [^{68}Ga]GaFAPI-46 was successfully produced with high radiochemical purity and yield using an IBA Synthera® Extension module. Gallium chloride was cyclotron produced on a medical cyclotron using a liquid target with an activity of 4.31 ± 0.36 GBq at the end-of-purification (EOP). Analytical methods were established and validated meeting Ph. Eur. standards. Fully GMP production was also validated in three consecutive batches, producing 2.50 ± 0.46 GBq of [^{68}Ga]Ga-FAPI-46 at the end-of-synthesis (EOS), with 98.94 ± 0.72% radiochemical purity by radio-HPLC. Quality was maintained for up to 3 h after the EOS. Production of [^{68}Ga]GaFAPI-46 was performed and validated using a standard medical cyclotron with liquid targets. Quality control parameters (*e.g.* sterility, purity, and residual solvents) conform to Eur. Phar. and shelf life of 3 h was established. Activity of [^{68}Ga]GaFAPI-46 produced is substantially higher than the one obtained with generators enabling a better response to the clinical need for this radiopharmaceutical.

Keywords: radiopharmaceuticals; cyclotron; liquid targets; FAPI-46

1. Introduction

Cancer-associated fibroblasts (CAFs) play an important role in the tumour microenvironment, exhibiting functions in tumour migration, growth, metastasis, progression, and resistance to chemotherapy [1]. Fibroblast activation protein (FAP) is a membrane-bound glycoprotein that is specifically overexpressed in activated fibroblast, including CAFs [2]. FAPI-46, in particular, has emerged as a very promising theranostic tool in cancer. When labelled with the positron-emitting gallium-68, FAPI-46 has showed improved tumour-to-organ ratios, allowing for high contrast PET imaging of multiple types of tumours [3]. In this context, there have been several attempts to optimize the GMP production and quality control of [^{68}Ga]GaFAPI-46, and its therapy counterpart [^{177}Lu]LuFAPI-46, to meet the increasing clinical demand for these radiopharmaceuticals [4–7].

Gallium-68 is available worldwide through the ^{68}Ge/^{68}Ga generator [8]. Despite being convenience, generators have limitations in terms of the maximum number of elutions and activity per elution, high cost, and the possibility of contamination with long-lived parent radionuclide germanium-68 (half-life 271 days) [9]. On the other hand, cyclotron production of gallium-68 takes advantage of

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the extensive network of medical cyclotrons available that can produce considerable amounts and perform consecutive production cycles [10]. The production of [^{68}Ga]GaFAPI-46 using gallium-68 from a cyclotron has not been reported, to our knowledge. The validation of its GMP production can help us to meet the growing demand for this radiopharmaceutical worldwide. In this work, we describe a method for the GMP automated synthesis and full validation of this process. We believe that this could serve as a roadmap for other laboratories that are trying to implement the routine synthesis of this radiopharmaceutical.

2. Results

2.1. [^{68}Ga]GaFAPI-46 synthesis

Gallium-68 was produced using a standard medical cyclotron (IBA Cyclone Kiube, Louvain-la-Neuve, Belgium) by the irradiation of a zinc-68 solution for 70 to 80 minutes. Purification and synthesis were performed on an IBA Synthera extension fully automated platform (Louvain-la-Neuve, Belgium). The starting activity at the EOP was 4.31 ± 0.36 GBq (n=3), and the peptide quantity was 50 µg/batch (11.67 ± 0.97 µg/GBq). All syntheses were successfully completed within 25 minutes after the EOP, and all quality control parameters were in line Phar. Eur. specifications. Notably, the highest activities in the final product vial, with approximately 2.5 GBq of [^{68}Ga]GaFAPi-46, produced comparable results in terms of radiochemical purity to the lower activity vials (Table 1). Table 2 summarizes the results obtained from Spreckelmeyer (2020) and Alfeimi (2022) studies, which used different synthesis modules and generator produced gallium-68 to synthetize [^{68}Ga]GaFAPI-46 [6,7]. In comparison to these studies, we achieved similar results in terms of radiochemical yield (RCY) and radiochemical purity (RCP), with values of 90.53% and 98.94% (by radio-High-Performance Liquid Chromatography, radio-HPLC), respectively, but with up to three times more activity at the EOS.

Table 1. Results of [^{68}Ga]GaFAPI-46 produced with gallium-68 from cyclotron. Total amount of peptide 50 µg, 5-minute reaction time at 90ºC.

Synthesis no	Starting Activity (GBq)	Amount of peptide (µg/GBq)	Radiochemical Yield (%)	Purity HPLC (%)	Purity TLC (%)
1	4.68	10.67	90.20	99.61	99.86
2	4.27	11.70	91.00	98.18	99.94
3	3.96	12.62	90.04	99.04	99.13
Mean ± SD	**4.31 ± 0.36**	**11.67 ± 0.97**	**90.53 ± 0.42**	**98.94 ± 0.72**	**99.64 ± 0.45**

Table 2. Comparison of reaction conditions, RCY, and RCP yielded from Spreckelmeyer and Alfeimi. Conditions corresponding to the synthesis using 50 µg of peptide and gallium-68 from generator.

	Spreckelmeyer *et al.* on MLPT	Spreckelmeyer *et al.* on ML eazy	Alfeimi *et al.* on Trasis EasyOne	Alfeimi *et al.* on Synthra	Alfeimi *et al.* on Scintomics
Reaction Temp.	95 ºC	98 ºC	90 ºC	90 ºC	90 ºC
Reaction time	10 min	10 min	4 min	4 min	4 min
RCY (%)	95.20 ± 1.40	89.70 ± 6.70	92.45	92.32	92.86

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RCP (%) HPLC	99.70	99.70	99.40	99.70	99.80
RCP (%) TLC	99.90	99.90	-	-	-

2.2. Validation of analytical methods

Validation was performed for the 3 key QC techniques used to assess the quality of [^{68}Ga]GaFAPI-46: HPLC, Thin Layer chromatography (TLC) and Gas chromatography (GC). Method validation and analysis for sterility was conducted by a certified outsourcing company (Microbios, Barcelona, Spain). pH and endotoxin analysis were performed as described in the relevant monographs of Ph. Eur. [11]. HEPES test was based on previously established procedures for other ^{68}Ga-based radiopharmaceuticals (*e.g.*, monograph of GALLIUM (^{68}Ga) PSMA-11 INJECTION).

2.2.1. HPLC, TLC and GC methods validation

HPLC is used to identify and quantify radiochemical impurities in the drug product (Figure 1). The radio-TLC technique is used to establish the radiochemical purity of [^{68}Ga]GaFAPI-46 (Figure 2), and GC to quantify the presence of ethanol in the final formulation. To validate the analytical methods, the following parameters were checked: accuracy, repeatability, selectivity/specificity, quantification limit (LOQ), linearity, and range [12]. A summary of the validation of HPLC, TLC and GC methods results can be seen in Table 3.

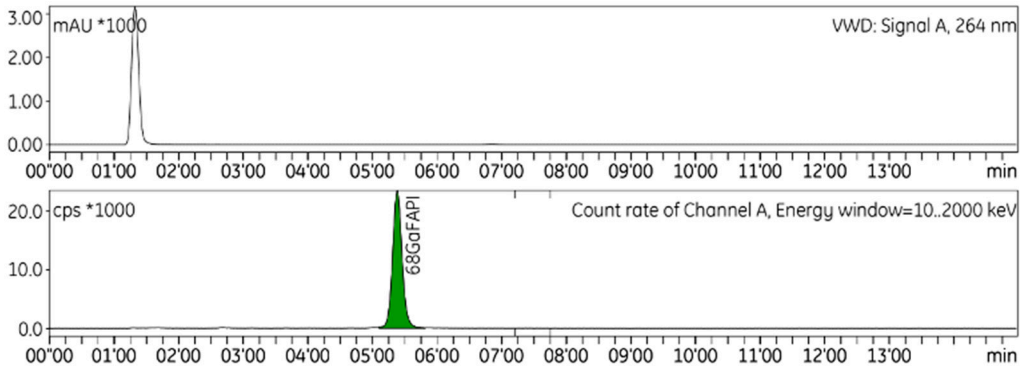

Figure 1. Representative radio-HPLC chromatogram of [^{68}Ga]GaFAPI-46.

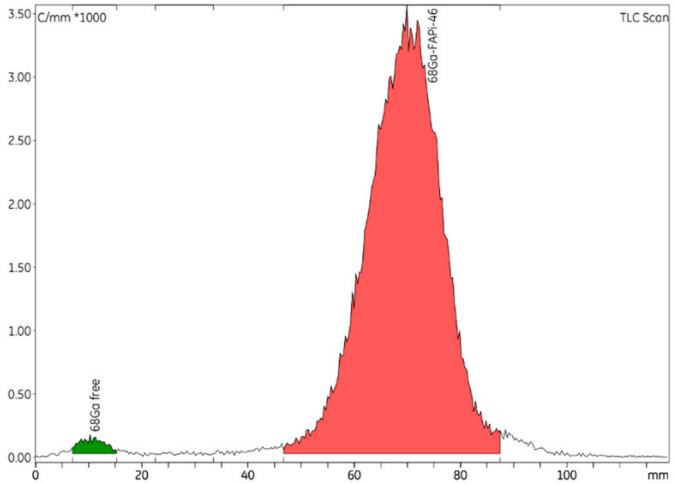

Figure 2. Representative radio-TLC chromatograms of [^{68}Ga]GaFAPI-46.

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Table 3. Summary of validation results of HPLC, TLC and GC methods.

Validation results summary of the HPLC method				
Test Parameter	**Acceptance criteria**		**Results**	
Repeatability	6 repetitions of [^{68}Ga]GaFAPI-46	RSD ≤ 5%	4.89	Table S1 (a)
	6 repetitions of [^{nat}Ga]GaFAPI-46	RSD ≤ 5%	3.40	Table S1 (b)
Specificity/ Selectivity	Resolution between peaks:			
	[^{68}Ga]GaFAPI-46	5.0 ≤ RT ≤ 6.0	5.37	Figure S1 (c)
	[^{nat}Ga]GaFAPI-46	5 ≤ RT ≤ 6	5.28	Figure S1 (b)
	[^{68}Ga]$GaCl_3$	2 ≤ RT ≤ 2	1.33	Figure S1 (a)
	RRT ([^{nat}Ga]GaFAPI-46/[^{68}Ga]GaFAPI-46)	0.9 ≤ RRT ≤ 1.1	0.98	
LOQ	S/N ratio ≥ 10	≤ 0.5 MBq/mL	10.70	Figure S3 (a)
Linearity	MBq/mL (5 concentrations)	R^2 ≥ 0.99	1.00	Figure S4 (a)
Range	Reported Value	0.13 - 99.77 MBq/mL		
Validation results summary of the TLC method				
Test Parameter	**Acceptance criteria**		**Results**	
Repeatability	6 repetitions of [^{nat}Ga]GaFAPI-46	RSD ≤ 0.2%	0.12	Table S2
Specificity/ Selectivity	Resolution between peaks:			
	[^{68}Ga]GaFAPI-46	R/F > 0.55	0.63	Figure S2 (b)
	[^{68}Ga]$GaCl_3$	R/F < 0.15	0.12	Figure S2 (a)
LOQ	S/N ratio ≥ 10	(0.17 MBq/mL)	23.1	Figure S3 (b)
Linearity	MBq/mL (5 concentrations)	R^2 ≥ 0.99	1.00	Figure S4 (b)
Range	Reported Value	0.15 - 47.00 MBq/mL		
Validation results summary of the GC method				
Test Parameter	**Acceptance criteria**		**Results**	
Repeatability	6 repetitions of [^{68}Ga]GaFAPI-46	RSD ≤ 5%	1.90	Table S3
LOQ	S/N ratio ≥ 10	(50 mg/10 mL)	247.70	Figure S3 (c)

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Linearity	50 - 2500 mg/10 mL (6 concentrations)	$R^2 \geq 0.99$	1.00	Figure S4 (c)
Range	Reported Value	50 - 2500 mg/10 mL		
Precision	6 repetitions of EtOH 2500 mg/10 mL	RSD ≤ 5%	3.88	
Accuracy	Spiked conc. 1500 mg/10 mL	≤ 10%	6.83	

2.3. Quality control

Quality control (QC) tests for [^{68}Ga]Ga-FAPI-46 were established based on the current requirements for other ^{68}Ga-based radiopharmaceuticals monographs (e.g., monograph of GALLIUM (^{68}Ga) PSMA-11 INJECTION). Table 4 displays the results of three exemplificative QC batches of [^{68}Ga]GaFAPI-46, which were also used to validate the synthesis process of the final drug product.

Table 4. Summary of the product specifications for [^{68}Ga]Ga-FAPI-46, and quality control test of three batches for process validation.

Tests	Method	Specifications	Results (n=3)
Appearance	Visual Inspection	Clear, colorless, or slightly yellow solution	**Comply**
pH	Potentiometric or strips	4 to 8	**6.5**
Identification			
Radionuclidic Identification – Energy photons γ	Gamma-ray spectrometry	The principal gamma photons have energies of 0.511 MeV and 1.077 MeV, and a sum peak of 1.022 MeV may be observed; peaks due to gamma photons with energy of 1.883 MeV may be observed.	**Comply**
Half-life	Ionization Chamber	61 min to 75 min	**67.6**
Chemical Purity			
HEPES	TLC	≤ 0.5 mg/10 mL	**Comply**
Radiochemical Purity			
[^{68}Ga]Ga-FAPI-46	radioHPLC	≥ 95%	**97.8**
Peak area of gallium-68 species RF <0.2	TLC (Radioactivity detector)	≤ 3%	**1.3**
Radionuclidic Purity			
Gallium-68	Gamma-ray spectrometry	≥ 98%	**99.8**
Gallium-66 and Gallium-67 [1,2]	Gamma-ray spectrometry	≤ 2%	**0.2**

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Other gamma-ray-emitting impurities [1,3]	Gamma-ray spectrometry	≤ 0.1%	**0.0**
Residual Solvents			
Ethanol [4]	GC-FID	≤ 2500 mg/10 mL	**732.1**
Biological Tests			
Endotoxin analysis	Direct inoculation	No evidence of growth should be found	**Comply**

[1] According to Ph. Eur., these tests are carried out after batch release for use. [2] Retain the preparation to be examined for at least 12h to allow the gallium-68 to decay to a level that permits the detection of gallium-66 and gallium-67. [3] Retain the preparation to be examined for at least 24h to allow the gallium-68 to decay to a level that permits the detection of impurities. Disregard the peaks due to the decay of gallium-66 and gallium-67. [4] According to Ph. Eur., maximum 2.5 g per administration assuming the density (2.2.5) to be 0.790 g/mL.

2.4. [^{68}Ga]GaFAPI-46 stability

The stability of [^{68}Ga]Ga-FAPI-46 in 10% (v/v) ethanol formulation at room temperature was tested up to 3 h by radio-HPLC, [^{68}Ga]Ga-FAPI-46 was not stable under those conditions at higher activities. During this time period, two radioactive side-products were detectable, with increased percentage at higher activities. The stability of [^{68}Ga]Ga-FAPI-46 was achieved by adding 500 mg of sodium ascorbate to the final formulation. Moreover, the radiochemical purity of all batches remained above 95%, stable over a 3h period of incubation at room temperature (RT), regardless of vial activity (Figure 3).

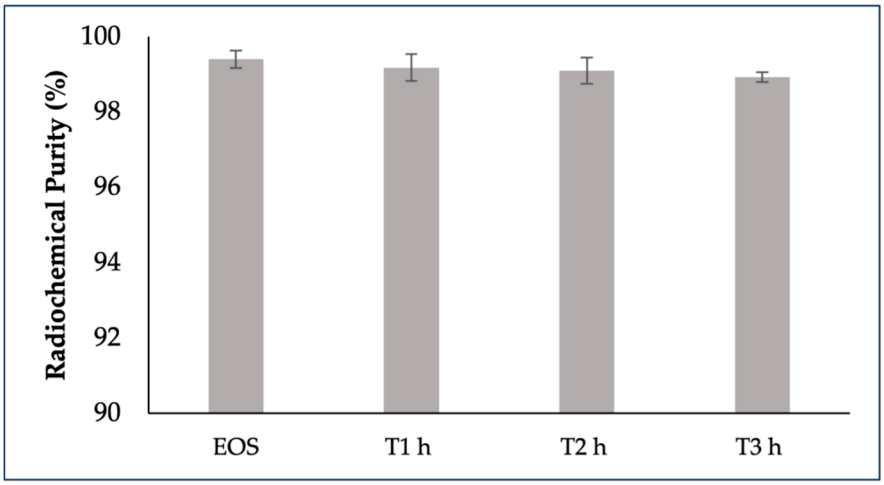

Figure 3. Stability of [^{68}Ga]GaFAPI-46 up to 3h after the end-of-synthesis. [^{68}Ga]GaFAPi-46 was formulated in NaCl 0.9% with 500 mg of sodium ascorbate.

3. Discussion

Typically, the synthesis of ^{68}Ga-based radiopharmaceuticals is performed using 1.85 GBq (from the generator elution). Protocols for fully automated production of [^{68}Ga]GaFAPI-46 using generators have been published with up to 1.7 GBq of [^{68}Ga]GaFAPi-46 [7] and, generally, over 90% radiochemical yield (Table 2). In this article, we present the results and validation of a fully automated synthesis method of [^{68}Ga]GaFAPI-46 produced from cyclotron. Furthermore, we have validated the specific QC analytical methods for this tracer.

When using gallium-68 from a cyclotron to produce [^{68}Ga]GaFAPi-46, the starting activities of gallium-68 can be as high as 5 GBq after purification. With the increasing demand for ^{68}Ga-based

 doi:10.20944/preprints202309.0931.v1

radiopharmaceuticals in the last decade, cyclotron production of gallium-68 enables a better response to the clinical doses of gallium-68 which are routinely necessary. The automated synthesis of [^{68}Ga]GaFAPI-46 using Synthera® Extension (Table 1) was found to be highly reproducible. Our results demonstrate that the use of 50 µg of FAPI-46 yields a similar radiochemical yield as in previously described studies [6,7], even with triple the activity.

The addition of sodium ascorbate in the final formulation of the product prevented radiolysis of the radiopharmaceutical. The formulation of [^{68}Ga]GaFAPI-46 in saline with 10% vol ethanol was found to be unstable. Although this effect has already been formerly described, it had not been observed to this extent. The use of ascorbic acid in the reaction has been reported to improve stability for ^{68}Ga- and ^{177}Lu-based radiopharmaceuticals [4,5,13]. Additionally, the use of sodium ascorbate in the final formulation has been shown to enhance stability in [^{177}Lu]LuFAPI-46. Consistent with these findings, the use of sodium ascorbate prevented degradation of [^{68}Ga]GaFAPI-46 over a 3h period, even at high activities of up to 3.0 GBq. Results of synthesis validation, summarized in Table 4, demonstrate that all the tested quality parameters were in accordance with the Phar. Eur.

4. Materials and Methods

The FAPI-46 precursor and the standard [^{nat}Ga]Ga-FAPI-46 were manufactured by ABX (Radeberg, Germany) and were made available, free of charge, by SOFIE Biosciences (Dulles, VA, USA). An aqueous stock solution of 1 mg/mL was prepared, and aliquots of 50 µg were stored at -15° C. All chemicals were of analytical grade, and the solvents for high-pressure liquid chromatography (HPLC) were purchased as HPLC grade. Enriched zinc-68 (66 mg/mL and 98.0% isotopic enrichment) for gallium-68 production, as well as all the chemicals and tubing kits for gallium-68 purification, were purchased from Fluidomica (Cantanhede, Portugal).

4.1. Irradiation and purification of [^{68}Ga]GaCl$_3$

A regular production of gallium-68 via cyclotron liquid target was achieved using the method described previously [10]. In summary, a solution of 66 mg/mL of zinc-68 was irradiated for a duration of up to 70 minutes using a Cyclone Kiube variable energy cyclotron (IBA; Louvain-la-Neuve, Belgium). Following the irradiation, the resulting target solution was transferred to a shielded hot-cell and automatically purified using the method formerly detailed [14,15].

4.2. Synthesis of [^{68}Ga]GaFAPI-46 using a Synthera® Extension synthesizer

For the fully automated synthesis of [^{68}Ga]GaFAPI-46 using IBA (Louvain-la-Neuve, Belgium) Synthera® Extension module, shown in Figure 1a, we used single-use labelling cassettes and reagent kits supplied by Fluidomica. The reagent kit included a SXC bound elute cartridge, a C18 plus short cartridge, HEPES buffer (0.5 M), saline, ethanol, and a sodium ascorbate vial (500 mg). The C18 cartridge required preconditioning with 10 mL of ethanol and 10 mL of water, and drying with air before use. Before galium-68 purification, the reaction mixture, consisting of 1 mL of 0.5 M HEPES buffer, 10 mg of ascorbic acid, and 50 µg of FAPI-46, was introduced into the reactor vial.

The initial step in the synthesis process involved concentrating the [^{68}Ga]GaCl$_3$ solution using an SCX cartridge and a peristaltic pump to prevent cross-contamination of the tubing system with free gallium-68. The loaded SCX cartridge was later eluted with a 5 M NaCl (in 0.05 M HCl) solution. To label [^{68}Ga]GaFAPI-46, the gallium-68 solution was mixed with 50 µg of FAPI-46 precursor and heated to 90o C for 5 minutes. Following the labelling reaction, the solution was passed through the C18 cartridge, eluted with a mixture of 2 mL water/ethanol (1:1) and filtered into the final product vial, which was infused with sodium ascorbate.

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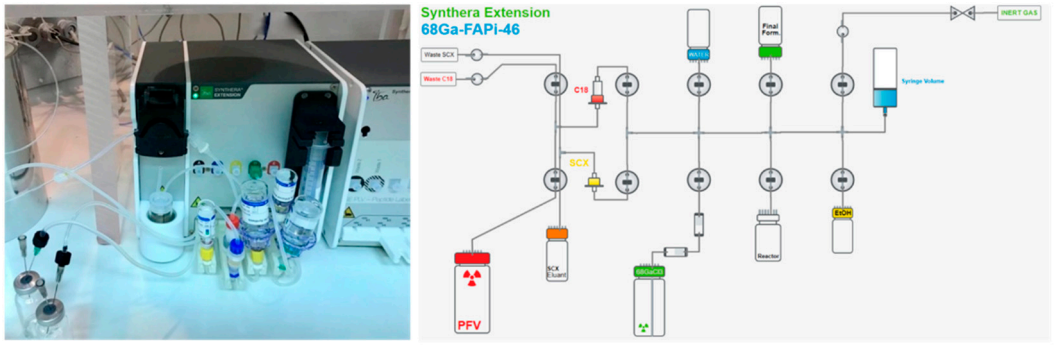

(a) (b)

Figure 4. Set up to produce [^{68}Ga]GaFAPI-46 on Synthera Extension Module. (**a**) Synthera Extension module with kit and reagents installed; (**b**) Layout scheme of the automatic module for the synthesis of [^{68}Ga]GaFAPI-46.

4.3. Radionuclidic identity and purity

4.3.1. HPGe Analysis

The radionuclidic purity (RNP) of gallium-68 at the end-of-beam (EOB) was determined through γ-spectroscopy of the final solution using a High Purity Germanium detector (HPGe), several hours after the EOB. The HPGe was calibrated with 154Eu and 133Ba radioactive sources and placed in a low-background shielding. γ-spectra were acquired using point-source-like samples with a dead-time below 4%. GammaVision (ORTEC Inc.) software was used to determine photopeak areas.

4.3.2. Half-life measurements

The half-life was determined by measuring, gallium-68 in the Ionization Chamber with a time interval of 5 min.

4.4. Radiochemical Purity and Identity

4.4.1. HPLC Analysis

The HPLC method used in this study was previously described by Eryilmaz et al. [5]. Tables 5 A and B specify the equipment and operating conditions used during all HPLC analyses.

Table 5. HPLC method used to identify and determine radiochemical impurities in the [^{68}Ga]GaFAPI-46 drug product. (a) Specifications of the HPLC equipment used, model Agilent 1260 Infinity II. (b) Operation conditions of the method.

	(a)	
Column		Avantor/ACE, ACE 3 C18, 3 µm, 150 x 3 mm S/N: A210625054
Detector		VWD 1260 Infinity II G7114A
Data acquisition software		Software Gina X
	(b)	
Wavelength	:	264 nm
Scintillation	:	Allow LOQ ≤ 0.05 MBq/mL

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Column temperature	:	Room temperature (not controlled)
Flow	:	0.6 mL/min
Injection volume	:	20 µL
Run time	:	15 min
Program	:	Time (min) / % Mobile phase A / %Mobile phase B 0.0 / **87** / **13** 15.0 / **87** / **13**
Mobile Phase A	:	Water/TFA = 1000/1 (v/v)
Mobile Phase B	:	Acetonitrile/TFA = 1000/1 (v/v)
Diluent	:	Water for injection

4.4.2. TLC Analysis

As described in the European Pharmacopeia (Eur. Phar.), an ammonium acetate (77 g/L):methanol (50:50 v/v) solution was used as the mobile phases for iTLC, and iTLC-SG strips were used as the stationary phases. The colloidal species of gallium-68 were detected at Rf < 0.1 and the product [^{68}Ga]GaFAPI-46 was detected at Rf > 0.5.

Table 6. TLC method used to determine radiochemical purity of [^{68}Ga]GaFAPI-46 drug product. (a) Specifications of the TLC equipment used, model miniGita; (b) Operation conditions of the method.

(a)			
Detector			Scintillation
Data acquisition software			TLC Control software, version 2.30
(b)			
Detector	Scintillation	:	Allow LOQ ≤ 0.5 MBq/mL
Others	Chromatographic paper	:	Agilent iTLC-SG
	Application volume	:	5 µL
	Elution length	:	80 mm (origin: 1.0 cm from the bottom end; elution front: 2.0 cm from the top end)
	Mobile Phase A	:	Ammonium acetate 1.0 M / methanol = 1/1 (v/v)
	Diluent	:	Water for injection

4.5. Residual Solvents

4.5.1. Ethanol

The presence of ethanol was evaluated by gas chromatography (GC) using an Agilent 6850 RaytestGmbh (Straubenhardt, Germany) GC system.

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4.5.2. HEPES

This system uses TLC aluminum foil as the stationary phase and a mixture of water and acetonitrile (25:75 v/v) as the mobile phase. A reference solution containing 200 µg of HEPES in 10 mL of water was eluted in the strip along with the sample and the strip is then exposed to iodine vapor for 4 min for HEPES detection.

Table 7. GC method used to quantify the presence of EtOH in the final drug product. Specifications of the GC equipment used, model 6850A, and operation conditions of the method.

Injector	
Mode	Split
Temperature	250 °C
Split ratio	15:1
Gas	Helium
Liner	Cone liner with glass wool, 4.0 mm ID, PN#5183-4647.
Oven	
Equilibrium time	0.00 min
Run time	15.0 min
Detector	
Temperature	260 °C
Mode	Constant Makeup
Makeup flow	30 mL/min (He)
Hydrogen flow	30 mL/min
Air flow	300 mL/min
Column (HP-Fast Residual Solvent; PN#1095V-420E or equivalent)	
Mode	Constant flow
Flow	3.0 mL/min
Length	30 m
Internal diameter	530 µm
Film Thickness	1.0 µm

4.6. Stability of [^{68}Ga]GaFAPI-46

The stability of [^{68}Ga]GaFAPI-46 in its final formulation, consisting of 10% EtOH (v/v) with ascorbic acid, was evaluated by HPLC and the stability measurements were quantified using the previously validated method. The protocol published by Fonseca et al. was followed with minor changes [16]. Aliquots were taken at different time points and measured using the HPLC method, up to three hours after the end-of-synthesis.

5. Conclusion

In this study, we demonstrated that [^{68}Ga]GaFAPI-46 can be produced according to GMP using liquid targets in a medical cyclotron, resulting in significantly higher production levels compared to ^{68}Ge/^{68}Ga generators. The high radionuclidic and radiochemical purity, as well as the stability of the final drug product, indicate that this method could serve as an alternative to the conventional gallium-68 generators. These findings provide a roadmap for future [^{68}Ga]GaFAPI-46 implementations aimed at meeting the routinely necessary clinical dose requirements.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1: Repeatability results of HPLC method for (a) [^{68}Ga]GaFAPI-46 and (b) [^{nat}Ga]GaFAPI-46; Table S2: Repeatability results of radioTLC method for [^{68}Ga]GaFAPI-46; Table S3: Repeatability results of GC method for ethanol content in [^{68}Ga]GaFAPI-46; Figure S1: LOQ representative chromatograms of HPLC; TLC and GC methods; Figure S2: Linearity plots of (a) HPLC, (b) TLC and (c) GC methods.

Author Contributions: Conceptualization, A.I.F., V.H.A. and F.A.; methodology, A.I.F. and V.H.A.; resources, I.H.; investigation, A.I.F.; writing—original draft preparation, A.I.F.; writing—review and editing, A.J.A., F.A. and V.H.A.; supervision—A.J.A., F.A. and A.F.; project administration, A.J.A. and F.A. All authors have read and agree to the published version of the manuscript.

Funding: This work was founded by the Portuguese Foundation for Science and Technology (FCT) through PhD grants (grant number PD/BDE/150681/2020 and PD/BDE/150331/2019) and by ICNAS-P.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article and supplementary material.

Acknowledgments: The authors gratefully acknowledge SOFIE Biosciences (Dulles, VA, USA) for providing the peptide for this study.

Conflicts of Interest: The authors declare no conflict of interest.

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