

Semi-automated [68Ga]Ga-FAPI-46 radiosynthesis using GalliAd® generator and MiniAIO® synthesizer: development and validation for PET imaging of fibroblast activation protein

Arnaud Deschavannes

arnaud.deschavannes@chu-lyon.fr

Hospices Civils de Lyon <https://orcid.org/0009-0002-5302-835X>

Nicolas Jacquet-Francillon

Hospices Civils de Lyon

Marie Piquemal

Hospices Civils de Lyon

Anthime Flaus

Hospices Civils de Lyon

Anthony Clotagatide

Hospices Civils de Lyon

Catherine Rioufol

Hospices Civils de Lyon

Emmanuel Odouard

Hospices Civils de Lyon

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Abstract

Background: Fibroblast activation protein (FAP) is a membrane-bound serine protease that is selectively overexpressed on cancer-associated fibroblasts (CAFs) in more than 90% of epithelial tumours. [^{68}Ga] ^{68}Ga labelled FAP inhibitors (FAPI) have emerged as promising PET tracers for oncological imaging and theranostic applications. [^{68}Ga] ^{68}Ga -FAPI-46 is a DOTA-chelated FAPI that demonstrates superior tumour retention compared to earlier FAPI compounds. We describe the development, optimisation, and pharmaceutical validation of a combination not previously reported for the production of this radiopharmaceutical: a semi-automated, one-step radiosynthesis of [^{68}Ga] ^{68}Ga -FAPI-46 without pre-purification of the generator eluate, performed at the hospital radiopharmacy of the Groupement Hospitalier Sud, Hospices Civils de Lyon, in the context of a clinical trial (EudraCT 2025-524897-40-00).

Radiolabelling was performed via a miniAIO automated synthesizer (Trasis) and a GalliAd [^{68}Ge]/[^{68}Ga] generator (IRE-Elit). The process - generator elution, direct radiolabelling at 95°C for 7 min in acetate buffer with L-ascorbic acid, HLB solid-phase purification and 0.22 μm sterilizing filtration - was performed in a single uninterrupted automated sequence. The quality control testings included release tests (visual appearance, final volume, radioactivity, pH, radiochemical purity by radio-TLC and radio-HPLC, radionuclidic identity, filter integrity, and bacterial endotoxin testing), post-release tests (radionuclidic purity and sterility), and validation batch-only tests (L-ascorbic acid and ethanol content). Analytical methods were validated as per ICH Q2(R2).

Results: All the validation batches met the predefined release and post-release specifications. The decay-corrected radiochemical yields ranged from 71.0% to 91.1%, with a total synthesis time of 20 min. Radiochemical purity exceeded 99.6% by radio-TLC and reached 100% by radio-HPLC at release, stayed $\geq 99.5\%$ after 3 hours. No radiolytic impurities were detected. Radionuclidic purity was confirmed with [^{68}Ge] ^{68}Ge breakthrough $<0.001\%$, and the measured half-life values (66–67 min) were consistent with those of [^{68}Ga] ^{68}Ga . Endotoxin levels were <1.0 IU/mL and all batches were sterile. Ethanol (5.4–7.1% v/v) and L-ascorbic acid (8.1–8.6 g/L) contents complied with predefined specifications. All analytical methods fulfilled the ICH Q2(R2) criteria, demonstrating adequate specificity, sensitivity and reproducibility.

Conclusions: A fully validated one-step automated radiosynthesis of [^{68}Ga] ^{68}Ga -FAPI46 was established via the use of a miniAIO synthesiser with a [^{68}Ge]/[^{68}Ga] generator. The process consistently produced a high-quality radiopharmaceutical suitable for clinical implementation and IMP submission in FAP-targeted PET imaging.

Trial registration : EudraCT No: 2025-524897-40-00; Clinical trials registry: NCT07313033 ; 15/01/2026.

1. Introduction

The tumour microenvironment (TME) is a complex ecosystem in which cancer-associated fibroblasts (CAFs) play pivotal roles in tumour growth, immune evasion, angiogenesis and extracellular matrix

remodelling (Park et al. 1999). CAFs are distinguished from resting fibroblasts by the selective surface expression of fibroblast activation protein (FAP), a type-II transmembrane serine protease with dipeptidyl peptidase and endopeptidase activities, that belongs to the DPP4 family (Hamson et al. 2014). FAP expression is minimal in most healthy adult tissues, with the exception of some organs (e.g., liver, pancreas), but is significantly upregulated in the activated stroma of > 90% of epithelial cancers, including pancreatic, colorectal, lung, breast and ovarian carcinomas (Kratochwil et al. 2019; Park et al. 1999). High stromal FAP expression has been identified as an independent negative prognostic marker in several tumour types, and its immunosuppressive role in the TME makes it an attractive target for both diagnostic and therapeutic purposes (Mona et al. 2022).

Targeting FAP with radiolabelled small-molecule inhibitors has emerged as a major advance in oncological molecular imaging. First, a quinoline-based structure capable of binding FAP with high affinity, that is suitable for [68Ga]Ga labelling was described (Lindner et al. 2018). Then, a first clinical PET/CT imaging with [68Ga]Ga-FAPI-02 and FAPI-04 demonstrated rapid tumour accumulation and low background activity in regions that typically confound [18F]FDG PET, such as the brain, liver and gastrointestinal tract (Loktev et al. 2018). FAPI-46, a quinoline-based DOTA-chelated inhibitor, was developed to improve FAP binding affinity and intratumoural retention compared to earlier compounds such as FAPI-02 and FAPI-04 (Loktev et al. 2019). While [18F]FDG reflects glucose metabolism and therefore accumulates in a wide range of malignant tissues, its physiological uptake in metabolically active organs such as the brain, myocardium, liver, and in inflammatory processes can limit lesion detectability and specificity (Boellaard et al. 2025). This difference is particularly relevant in histological subtypes with low or heterogeneous FDG avidity, such as invasive lobular carcinoma, certain mucinous tumours, and some low-grade or indolent malignancies, where [18F]FDG PET may underestimate disease extent or miss lesions altogether (Abbasi et al. 2025).

Breast cancer represents a particularly relevant indication for FAPI-targeted PET imaging. CAFs are abundant in the stroma of breast tumours, where FAP overexpression is correlated with poor prognosis and an immunosuppressive TME (Kratochwil et al. 2019). Invasive lobular carcinoma (ILC), which accounts for 5–15% of all breast cancers, is characterized by discohesive tumour cells dispersed through a rich in FAP-positive CAFs stroma, and displays consistently low [18F]FDG uptake (Gilardi et al. 2023; de Mooij et al. 2023; Sahin et al. 2024).

These findings directly motivated the present clinical trial (EudraCT 2025-524897-40-00; NCT07313033 ; FAPI-CL): “[68Ga]Ga-FAPI-46 PET/CT for the Diagnosis of Metastatic Lesions in Patients with Lobular Breast Cancer”, sponsored by the Hospices Civils de Lyon, which evaluates [68Ga]Ga-FAPI-46 PET/CT as an extension staging tool in patients with ILC for whom [18F]FDG PET/CT had insufficient diagnostic performance.

Several automated radiosynthesis processes for [68Ga]Ga-FAPI-46 have been reported on different synthesizers, including the Trasis AiO (Da Pieve et al. 2022), the iPHASE MultiSyn (Morandau et al. 2024) and the GAIA module (Rubira et al. 2024) which use two different types of [68Ga]Ga generators

(GalliAd, IRE-Elit and GalliaPharm, Eckert & Ziegler). The key validation study by Paty et al. defined optimal labelling conditions - notably the role of L-ascorbic acid and the reaction temperature – which formed the basis of our protocol (Paty et al. 2024). Direct use of the raw generator eluate without cation-exchange pre-purification was feasible for three different synthesizers, simplifying the workflow without compromising radiochemical quality (Alfteimi et al. 2022).

Currently, the European Pharmacopoeia does not include a monograph for [68Ga]Ga-FAPI-46, and no industrially manufactured kit with marketing authorization is available. Consequently, this radiopharmaceutical can only be administered in clinical trials as an investigational medicinal product. In France, [68Ga]Ga-FAPI-46 has no marketing authorization to date, making the preparation and submission of an investigational medicinal product dossier (IMPD) to the Agence nationale de sécurité du médicament et des produits de santé (ANSM) and the European Medicines Agency (EMA) mandatory prior to first use.

The objective of this work was therefore to achieve full pharmaceutical validation of the [68Ga]Ga-FAPI-46 production process to support IMPD submission and obtain authorization for its clinical use in patients enrolled in the FAPI-CL trial. This is the first published report describing the complete pharmaceutical validation of a one-step automated radiosynthesis of [68Ga]Ga-FAPI-46 using the specific combination of the miniAIO synthesizer directly connected to the GalliAd [68Ge]/[68Ga] generator, without pre-purification of the generator eluate, performed in compliance with Good Radiopharmacy Practices (ANSM 2023) and ICH Q2(R2) guidelines (ICH Q2 2024).

2. Materials and Methods

2.1. Radiopharmaceutical and precursor

[68Ga]Ga-FAPI-46 (also referred to as [68Ga]GaGEH300079; CAS : 2374784-98-0 ; MW 950 g/mol; $C_{41}H_{54}F_2GaN_{11}O_9$) is produced via DOTA-mediated chelation of the FAPI-46 precursor (DOTA-FAPI-46; CAS 2374782-04-2; MW 885.96 g/mol; 50 µg per vial). The precursor was supplied by SOFIE (Dulles, USA) and manufactured by ABX (Radeberg, Germany).

[68Ga]Ga is obtained as [68Ga]GaCl₃ by elution of a GalliAd [68Ge]Ge/[68Ga]Ga generator (MA 3400955057901 ; IRE-Elit, Fleurus, Belgium) using 1.1 mL of 0.1 mol/L sterile ultrapure HCl. Ultrapure reagents (sodium acetate EMSURE, L-ascorbic acid for analysis, water for analysis EMSURE, and anhydrous ethanol; Merck/Carlo Erba) are employed throughout the process to minimize trace metal contamination that could compete with [68Ga]Ga during DOTA chelation (Cusnir et al. 2019).

2.2. Synthesis equipment and environment

Radiosynthesis was performed via a miniAIO automated synthesizer (Trasis, Ans, Belgium), directly connected to the GalliAd [68Ge]Ge/[68Ga]Ga generator, controlled by Trasis Supervision software (Fig. 1). A single-use cassette (ref. F0491800, Trasis, Ans, Belgium) integrating two six-way valve

manifolds, a sterile reaction vial, an Oasis HLB (Hydrophilic-lipophilic balance) extraction cartridge and a 2-piece syringe was used for each production. The cassette was installed in a shielded class A hot cell within a class C environment, in compliance with Good Radiopharmacy Practices (ANSM 2023). All synthesis steps were performed in a closed system.

2.3. Radiosynthesis without pre-purification (Fig. 2)

This semi-automated, one-step radiosynthesis of $[^{68}\text{Ga}]\text{Ga-FAPI-46}$ without pre-purification of the generator eluate approach, validated on other platforms (Da Pieve et al. 2022; Morandea et al. 2024; Paty et al. 2024; Rubira et al. 2024), simplifies the workflow and reduces the synthesis time. The sequence comprised: (1) transfer of 1 ml of buffered FAPI-46 solution (50 μg of FAPI-46 in 0.2 mol/L aqueous sodium acetate solution) to the reaction vial; (2) HLB cartridge sequentially conditioned with 5 ml of ethanol and 5 ml of water for injection (WFI); (3) addition of 1.5 ml of 0.9% NaCl containing L-ascorbic acid (0.07 mol/l) into the reaction vial; (4) generator elution with 1.1 ml of 0.1 mol/l HCl and direct transfer of the raw $[^{68}\text{Ga}]\text{GaCl}_3$ eluate to the reaction vial; (5) radiolabelling at 95°C for 7 min; (6) HLB solid-phase purification; (7) sequential rinsing with ethanol (0.9 ml), WFI (2.0 ml) and L-ascorbic acid solution (q.s. 11.1 ml, 0.07 mol/l in 0.9% NaCl); and (8) sterilizing filtration through a 0.22 μm Millex filter into a sterile 11.5 ml type I glass vial (ref. SV-10E, Huayi Isotopes Company, Jiangsu, China). The total synthesis time was approximately 20 min. To control zinc(II) accumulation in the eluate – a known source of competitive inhibition of $[^{68}\text{Ga}]\text{GaCl}_3$ complexation (Cusnir et al. 2019) - a discarded cleaning elution of the generator was performed within 24 hours before each production run.

2.4. Final product composition

Each batch consisted of 11.1 ml of an isotonic injectable solution containing: 50 μg of FAPI-46 precursor complexed with $[^{68}\text{Ga}]\text{GaCl}_3$ at 30–70 MBq/ml, 0.9 ml of ethanol, 2.0 ml of WFI, 88 mg of L-ascorbic acid, and 0.9% NaCl to volume. The product was stored at room temperature (18–25°C), with a shelf-life of 3 hours post-synthesis.

2.5. Quality control specifications

Eight release tests and four post-release tests were performed in accordance with the European Pharmacopoeia monographs 0125 (Ph. Eur. 0125), 2464 (Ph. Eur. 2464), 3044 (Ph. Eur. 3044) and 20601 (Ph. Eur. 20601) (Table 1). Release with specifications tests included: visual appearance (clear and colourless), final volume (11.1 ml $\pm$ 10%), radioactivity (333–777 MBq), pH (4–7), RadioChemical Purity (RCP) by radio-TLC (Thin Layer Chromatography) and radio-HPLC (High-performance liquid chromatography) ($\geq$ 95.0%), radionuclidic identity by half-life ($\pm$ 10%) and gamma spectrometry (511 and 1077 keV), bacterial endotoxin (< 175 IU/dose; < 15.8 IU/ml), and filter integrity (leak < 500 $\mu\text{L}/\text{min}$). Post-release tests and specifications included assessments of radionuclidic purity ($[^{68}\text{Ge}]\text{Ge}$ < 0.001%), sterility and - for validation batches only - L-ascorbic acid (< 11 g/L) (ANSES) and ethanol content (< 10% v/v; < 78.9 mg/ml).

Table 1
Quality control specifications for [68Ga]Ga-FAPI-46.

Test	Method	Specification	Timing
Visual appearance	Visual inspection	Clear, colourless to slightly yellow, particle-free	Release
Final volume	Visual/graduation	11.1 ml $\pm$ 10%	Release
Radioactivity	MEDI 405 dose calibrator	333–777 MBq	Release
pH	pH paper	4–7	Release
RCP (radio-TLC)	ITLC-SG / acetate buffer:methanol (50:50)	[68Ga]Ga-FAPI-46 $\geq$ 95.0%; [68Ga]GaCl ₃ +colloids $\leq$ 5.0%	Release
RCP (radio-HPLC)	HPLC	[68Ga]Ga-FAPI-46 $\geq$ 95.0%; impurities $\leq$ 5.0%	Release
Radionuclidic identity	Half-life + gamma spectrometry (HPGe)	67.71 min $\pm$ 10%; 511 keV and 1077 keV	Release
Filter integrity	miniAIO automated test	Leak < 500 μ L/min at 80% bubble point	Release
Bacterial endotoxins	Kinetic colorimetric LAL (Endosafe nexgen-PTS)	< 175 IU/dose (< 15.8 IU/ml)	Release
Radionuclidic purity	Gamma spectrometry (HPGe) at t0 and t48h	[68Ge]Ge < 0.001%	Post-release
Sterility	(TSB + TGA, 14 days)	No microbial growth	Post-release
L-ascorbic acid	UPLC-electrochemistry (COFRAC lab)	< 11 g/L	Validation batch only
Ethanol content	Spectrophotometry (COFRAC lab)	< 78.9 mg/ml (< 10% v/v)	Validation batch only

2.6. Analytical method description and validation

Radio-TLC was performed via ITLC-SG strips with ammonium acetate (1 mol/L) / methanol (50:50, v/v) as the mobile phase; chromatograms were acquired using a Rita star radiochromatograph (Raytest, Straubenhardt, Germany). Radio-HPLC and UV-HPLC analyses were carried out on an Acquity H-Class UPLC system (Waters, Saint-Quentin-en-Yveline, France) equipped with a BEH C18 column 1.7 μ m, 2.1 $\times$ 50 mm (ref 186002350, Waters, Saint-Quentin-en-Yveline, France) and coupled to a NaI detector (HERM LB 500, Berthold, Bad Wildbad, Germany). The mobile phase consisted of water (A) and acetonitrile (B) delivered at a flow rate of 0.6 ml/min, with the following gradient: 0–9.0 min, linear gradient from 95% A / 5% B to 70% A / 30% B; 9.0–9.5 min, isocratic hold at 70% A / 30% B; 9.5–10.0 min, return to initial conditions (95% A / 5% B); followed by re-equilibration from 10.0 to 15.0 min at 95% A / 5% B. The retention time (t_R) identity was confirmed by comparison with the non-radioactive reference standard [natGa]Ga-FAPI-46 using UV detection at 280 nm. A radiolytic impurity, [68Ga]Ga-IMP1, was

characterized by performing the synthesis in the absence of an antioxidant. Analytical methods were validated in accordance with ICH Q2(R2) (ICH Q2 2024) for radio-TLC, radio-HPLC and UV-HPLC analyses, including assessment of specificity (resolution factor $R_s > 1.5$), linearity ($R^2 > 0.99$), limits of detection (LOD) and quantification (LOQ), repeatability ($n = 6$; Coefficient of variation (CV) $< 2\%$), robustness (CV $< 2\%$) and accuracy.

3. Results

3.1. Radiosynthesis and batch outcomes

Three consecutive validation batches were manufactured between October and November 2025 (Table 2). Starting activities ranged from 1133 to 1188 MBq. The decay-corrected radiolabelling yields were 71.0%, 76.0% and 91.1% for batches 1, 2 and 3 respectively. The total synthesis time was approximately 20 min. The appearance was clear and colourless for all batches. The measured pH values were 4.5, 5.0 and 4.0 for batches 1, 2 and 3, respectively. All batches met predefined release and post-release specifications.

Table 2
Results of the three [⁶⁸Ga]Ga-FAPI-46 validation batches

Parameter	Batch 1 (30/10/2025)	Batch 2 (31/10/2025)	Batch 3 (04/11/2025)
Batch number	N10025124390	N10025186586	N10025340675
Starting activity (MBq)	1155	1133	1188
Final activity (MBq)	666.7	708.8	662.5
Decay-corrected yield (%)	71.0	76.0	91.1
Appearance	Clear, colourless	Clear, colourless	Clear, colourless
pH	4.5	5.0	4.0
Half-life (min)	67	66	66
[⁶⁸ Ge]Ge (%)	< 0.001	< 0.001	< 0.001
RCP by TLC at t0h (%)	99.75	99.67	99.84
RCP by TLC at t3h (%)	99.50	99.50	99.74
RCP by radio-HPLC at t0h (%)	100	100	100
RCP by radio-HPLC at t3h (%)	100	100	100
Endotoxins (IU/ml)	< 1.0	< 1.0	< 1.0
Sterility	No microbial growth	No microbial growth	No microbial growth
Ethanol (mg/ml)	55.6 (7.1%)	51.7 (6.6%)	42.4 (5.4%)
L-ascorbic acid (g/L)	8.2	8.6	8.1

3.2. Radiochemical purity

Radio-TLC clearly resolved [⁶⁸Ga]Ga-FAPI-46 (Retardation factor (R_f) = 0.50–0.55) from [⁶⁸Ga]GaCl₃ and [⁶⁸Ga]Ga-colloids (R_f = 0.00), with a mean R_s = 3.79 ± 0.56 (n = 3), well above the 1.5 acceptance criterion. RCP by radio-TLC exceeded 99.67% at t = 0 h and remained above 99.50% at t = 3 h in all batches. By radio-HPLC, the product peak eluted at t_R = 4.46 ± 0.09 min. The radiolysis-derived impurity [⁶⁸Ga]Ga-IMP1 was not detected in any production batch, confirming the efficacy of L-ascorbic acid. RCP by radio-HPLC was 100% in all batches at both t = 0 h and t = 3 h.

3.3. Radionuclide identity and purity

Gamma spectrometry confirmed photon peaks at 511 keV and 1077 keV in all batches. Measured half-lives were 66–67 min (specification: 61.0–74.5 min). [⁶⁸Ge]Ge breakthrough was < 0.001% in all batches, as determined by delayed gamma spectrometry at t ≥ 48 h.

3.4. Stability over 3 hours

Visual appearance, pH (4.0–5.0) and RCP remained within specifications throughout the 3-hour storage period at room temperature, vial inverted. Container-content interaction (e.g., adsorption to vial walls) deviations ranged from – 2.1% to + 4.3%, all within the $\pm$ 5% acceptance criterion. RCP by radio-TLC remained above 99.50% at t = 3 h in all batches. RCP by radio-HPLC was 100% in all batches at both and t = 3 h.

3.5. Microbiological quality

Endotoxin levels were < 1.0 IU/ml in all batches (specification: < 15.8 IU/ml). Sterility tests showed no microbial growth in any batch.

3.6. Excipient content

Ethanol concentrations were 55.6, 51.7 and 42.4 mg/ml (5.4–7.1% v/v), all below the 78.9 mg/ml limit. L-ascorbic acid concentrations were 8.1–8.6 g/L, which is consistent with the theoretical value of 8.8 g/L and below the 11 g/L specification.

3.7. Analytical method validation

All acceptance criteria per ICH Q2(R2) (ICH Q2 2024) were met (Table 3). Specificity ($R_s > 1.5$), linearity ($R^2 \geq 0.99$), LOD, LOQ, repeatability (CV < 2%), robustness (CV < 2%) and accuracy were confirmed for all three analytical methods detailed in Table 3.

Table 3
Summary of analytical method validation results.

Parameter	Radio-TLC	Radio-HPLC	HPLC-UV (cold reference)
Specificity	3.79 $\pm$ 0.56 (> 1.5)	1.51–1.73 (> 1.5)	1.51–1.56 (> 1.5)
Linearity (R^2)	0.9904	0.9998	0.9944
LOD	0.0005 MBq/ml	0.04 MBq/ml	0.0016 μ g/ml
LOQ	0.0016 MBq/ml	0.44 MBq/ml	0.0049 μ g/ml
Repeatability (CV)	0.03% (RCP)	0.00% (RCP); 1.93% (tR)	0.91% (AUC); 0.53% (tR)
Robustness (CV)	0.00%	0.00%	0.04%
Accuracy	Confirmed	Confirmed	Confirmed

4. Discussion

We report the development, optimisation and full pharmaceutical validation of an automated one-step radiosynthesis of [68Ga]Ga-FAPI-46 performed on a miniAIO platform directly coupled to a GalliAd [68Ge]Ge/[68Ga]Ga generator in a hospital radiopharmacy setting, in compliance with Good

Radiopharmacy Practices (ANSM 2023) and ICH Q2(R2) (ICH Q2 2024). The resulting process provides a fully documented framework suitable for inclusion in an IMPD for clinical trial authorization by ANSM and the EMA.

This is the first study to describe and validate this specific instrumentation configuration. The integrated one-step approach - combining generator elution, radiolabelling and HLB purification within a single automated sequence - reduces synthesis time to approximately 20 minutes for total synthesis while limiting operator exposure and manual interventions. The performance of this streamlined process was consistent with previously reported methods using alternative synthesis modules and [68Ge]Ge/[68Ga]Ga generators (Da Pieve et al. 2022; Morandea et al. 2024; Paty et al. 2024; Rubira et al. 2024).

Furthermore, performing a cleaning elution of the generator within 24 hours prior to each synthesis effectively controlled zinc(II) accumulation in the eluate (Cusnir et al. 2019), thereby preventing competitive chelation with the DOTA macrocycle and limiting the formation of the chemical impurity IMP2 (tentatively identified as zinc(II)-FAPI-46 (Paty et al. 2024)).

The complete absence of the radiolysis impurity [68Ga]Ga-IMP1 across all validation batches confirms the efficacy of the dual L-ascorbic acid strategy - added during radiolabelling and in the final formulation and confirmed in other validated processes (Da Pieve et al. 2022; Morandea et al. 2024; Paty et al. 2024; Rubira et al. 2024). This oxidised species – eluting at ~ 3.51 min by radio-HPLC, reflecting greater hydrophilicity relative to the parent compound – formed rapidly under antioxidant-free conditions, as evidenced by a dedicated synthesis performed without L-ascorbic acid (Fig. 3). The dual addition of ascorbic acid - first during the radiolabelling step to limit radiolytic degradation of the precursor, and then in the final formulation to maintain stability throughout the 3-hour shelf life - resulted in complete suppression of this impurity to undetectable levels. This second addition is necessary, as ascorbic acid is not retained by the HLB cartridge during solid-phase extraction and is consequently removed in the waste fraction (Verma et al. 1996).

In addition to impurity control, the quantitative analysis of excipients represents a critical aspect of pharmaceutical validation in the absence of a dedicated pharmacopoeial monograph for [68Ga]Ga-FAPI-46. Ethanol concentrations remained well below the commonly accepted limit of 10% (v/v) for parenteral administration, ensuring acceptable tolerability and compliance with requirements for injectable preparations (Ph. Eur. 0125). Similarly, L-ascorbic acid concentrations were consistent with the theoretical formulation and remained below the predefined specification, supporting both antioxidant efficacy and patient safety. Notably, the total administered amount per vial remains within the nutritional reference values established for daily intake, thereby minimizing any potential toxicological concern (ANSES). These results highlight the importance of controlling not only active radiochemical parameters but also formulation excipients, particularly in the context of investigational radiopharmaceuticals where no authorized formulation exists.

The variability observed in decay-corrected radiolabelling yields across the three batches warrants contextualization. Batches 1 and 2 were synthesized using a chemical-grade precursor rather than a Good Manufacturing Product (GMP) grade material. Such chemical-grade peptides may contain higher levels of metallic impurities (e.g., iron, zinc, copper), which can compete with $[^{68}\text{Ga}]\text{GaCl}_3$ for coordination with the DOTA macrocycle (Velikyan et al. 2008), thereby reducing labelling efficiency. In contrast, batch 3, produced with a GMP-grade precursor supplied by ABX, achieved a higher yield of 91.1%. Despite this variability, the yields obtained for batches 1 and 2 remained sufficient to prepare the intended number of patient doses, and all batches complied with the predefined quality specifications. Nonetheless, these findings highlight the critical importance of using pharmaceutical-grade, certified precursors and of defining precursor grade as a key critical quality attribute.

The analytical method validation (ICH Q2 2024) confirmed the suitability of the quality control strategy for routine clinical use. The Rs between $[^{68}\text{Ga}]\text{Ga-FAPI-46}$ and its impurities exceeded 1.5 in all validation experiments for radio-TLC, radio-HPLC and UV-HPLC, meeting the specificity threshold recommended by the European Pharmacopoeia for chromatographic separation (Ph. Eur. 2024b). All methods showed excellent linearity, repeatability for radio-TLC and radio-HPLC were well within the acceptance criterion. LOD values were also below the specified impurity levels, confirming adequate sensitivity for product release.

A shelf-life of 3 hours post-synthesis was established based on stability data confirming maintenance of all quality parameters (RCP, pH, appearance) within specifications throughout this period. This duration is appropriate for same-day clinical PET imaging as the 67.71-minute half-life of $[^{68}\text{Ga}]\text{Ga}$. The 3-hour shelf-life thus represents a conservative, quality-driven boundary that satisfies both radiophysical constraints and pharmaceutical release criteria, while remaining operationally compatible with hospital scheduling.

The process described here directly supports the clinical trial FAPI-CL (EudraCT 2025-524897-40-00), which evaluated $[^{68}\text{Ga}]\text{Ga-FAPI-46}$ PET/CT for the diagnosis of metastatic lesions in patients with ILC. The growing theranostic interest in FAPI-46 evidenced by clinical experience further reinforces the value of this radiopharmaceutical as a theranostic tool beyond purely diagnostic applications.

Limitations of this study include the small number of validation batches ($n = 3$). While this aligns with regulatory expectations for IMPD, it may not fully reflect process variability across the generator's lifetime. Continued monitoring will be performed throughout operation, and additional batch data will be collected during the clinical trial.

5. Conclusion

A fully validated, semi-automated one-step hospital radiopharmacy process for $[^{68}\text{Ga}]\text{Ga-FAPI-46}$ was established at the Hospices Civils de Lyon, via a miniAIO synthesizer (Trasis) and a GalliAd $[^{68}\text{Ge}]\text{Ge}/[^{68}\text{Ga}]\text{Ga}$ generator (IRE-Elit). This specific instrumentation combination, not previously described for this radiopharmaceutical, consistently delivers a pharmaceutical-grade product. This

process provides a transferable and regulatory-compliant template for hospital-based production of [68Ga]Ga-FAPI-46, in direct support of the clinical trial EudraCT 2025-524897-40-00 targeting FAP-expressing malignancies including lobular breast cancer.

The theranostic potential of FAPI-46 continues to expand through the development of therapeutic analogues labelled with β - or α emitting radionuclides. In this context, standardised production protocols for the diagnostic counterpart [68Ga]Ga-FAPI-46 are essential to support patient selection, dosimetry and longitudinal treatment monitoring within theranostic frameworks.

Abbreviations

ANSM

Agence nationale de sécurité du médicament et des produits de santé

CAF

Cancer-associated fibroblasts

CV

Coefficient of variation

EMA

European Medicines Agency

FAP

Fibroblast activation protein

FAPI

FAP inhibitors

GMP

Good Manufacturing Practice

HLB

Hydrophilic-lipophilic balance

HPLC

High-performance liquid chromatography

ILC

Invasive Lobular Carcinoma

IMPD

Investigational medicinal product dossier

LOD

Limit of detection

LOQ

Limit of quantification

RCP

Radiochemical purity

R_f

Retardation factor

Rs
Resolution
TLC
Thin Layer Chromatography
TME
Tumour microenvironment
tR
Retention time
WFI
Water for injection

Declarations

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Author contributions

AD contributed to the conception and design of the work, the acquisition, analysis, and interpretation of data, and drafted the manuscript. CR, AC and AF revised the manuscript. MP and EO contributed to the conception and design of the work and revised the manuscript. All authors have approved the submitted version and have agreed to be personally accountable for their own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even those in which they were not personally involved, are appropriately investigated, resolved, and documented in the literature.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

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

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Figures

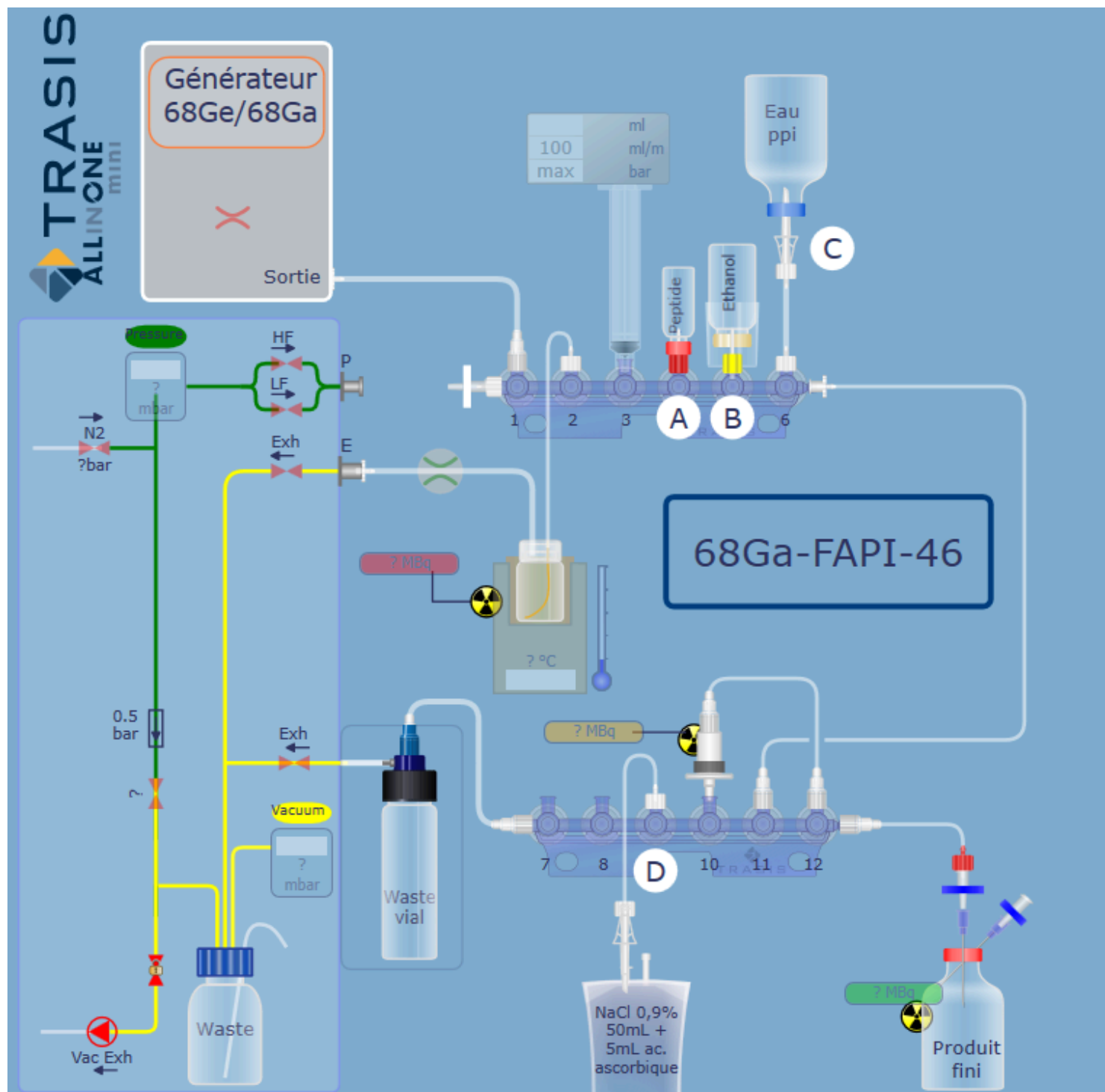


Figure 1

Schematic representation of the automated synthesis of [68Ga]Ga-FAPI-46

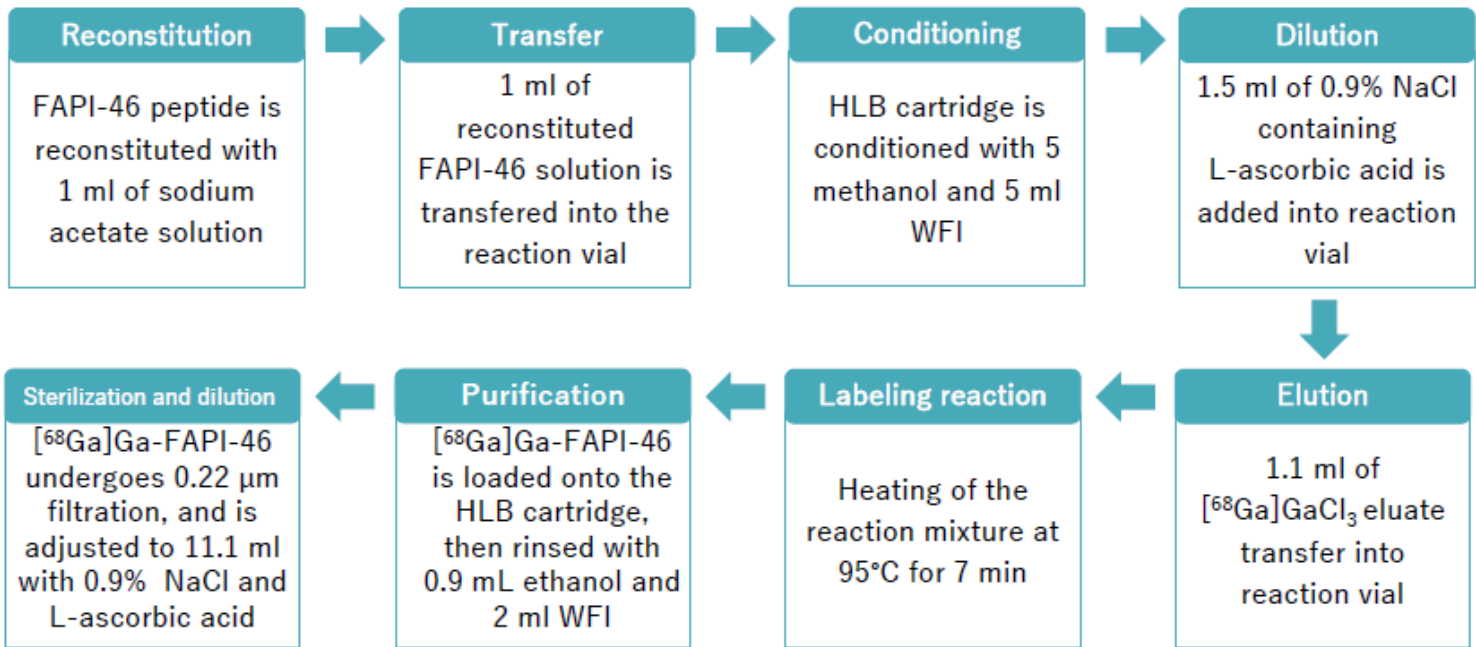


Figure 2

From reconstitution to purification - schematic overview of [68Ga]Ga-FAPI-46 labeling

HPLC-radio

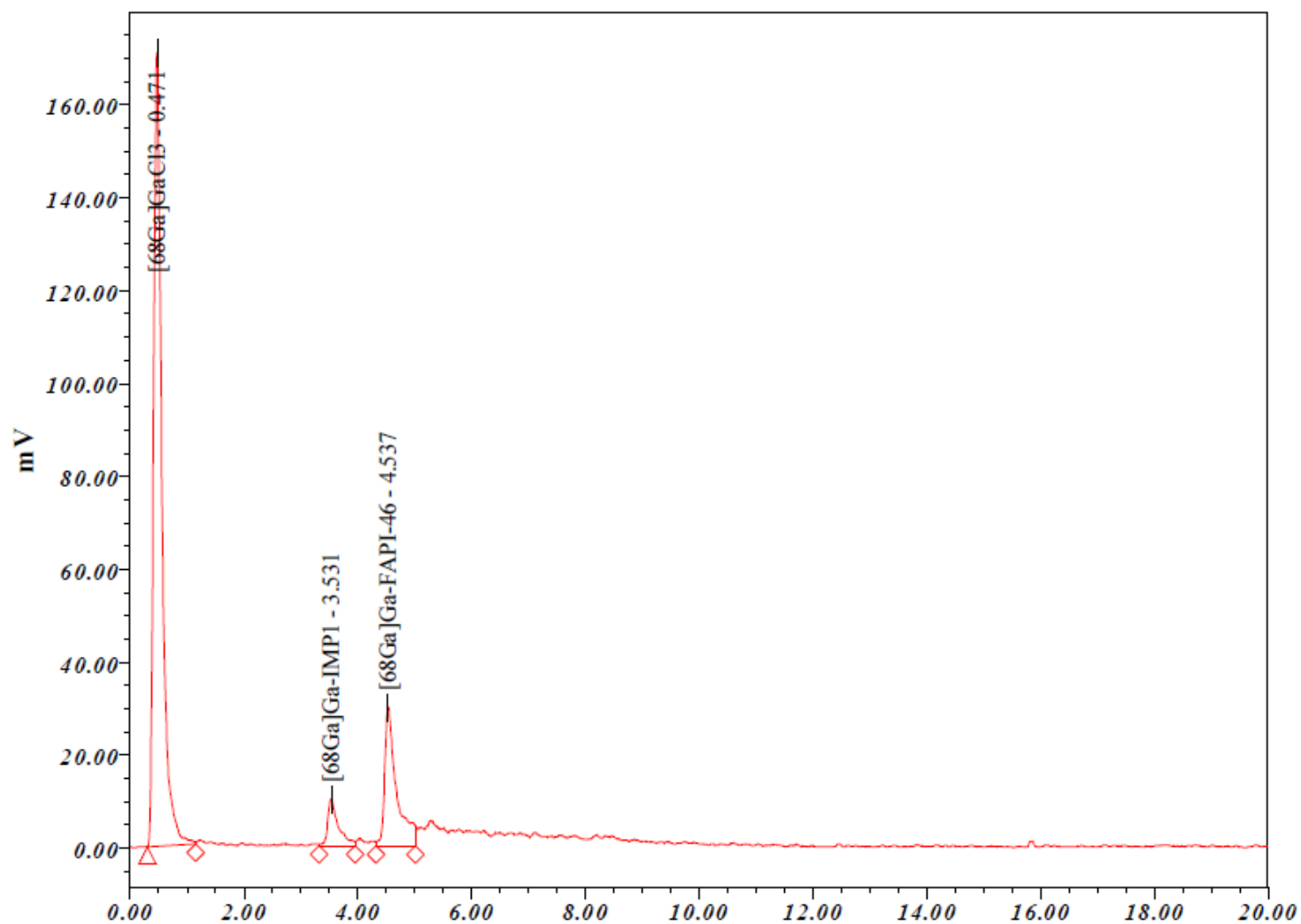


Figure 3

Characterization of the impurity $[^{68}\text{Ga}]\text{Ga-IMP1}$ by HPLC-radio