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Optimization of Manual Radiolabeling of [^{68}Ga]Ga-DOTA-FAPI-46 Based on ^{68}Ge/^{68}Ga Generator

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[68Ga]Ga-DOTA-FAPI-46; Radiolabeling; PET Imaging; Radiochemical Yield; Manual Synthesis; Fibroblast Activation Protein

Abstract

Cancer diagnosis increasingly requires molecular imaging agents with high tumor specificity and rapid biodistribution. [^{68}Ga]Ga-DOTA-FAPI-46 is a promising PET radiopharmaceutical that targets fibroblast activation protein (FAP); however, limited access to automated synthesis modules necessitates the development of reliable manual radiolabeling protocols. This study aimed to determine the optimal pH and temperature conditions for manual [^{68}Ga]Ga-DOTA-FAPI-46 radiolabeling using a ^{68}Ge/^{68}Ga generator and to evaluate its pharmaceutical quality. A 3 × 2 full factorial design was employed, combining three sodium acetate buffer pH levels (3.7, 4.0, and 4.5) with two reaction temperatures (95°C and 105°C), with each condition performed in triplicate. Radiochemical yield and purity were assessed, along with serum-free and human serum stability, sterility, and bacterial endotoxin levels. The optimal condition was pH 4.0 at 105°C for 10 minutes, producing a radiochemical yield of 87.516% ± 0.601%. pH significantly affected radiochemical yield, whereas temperature alone had no significant effect; however, their interaction was significant ($F = 17.531$; $p < 0.001$; $\eta^2 = 0.745$). Radiochemical purity exceeded 99% and remained above 95% during three-hour stability testing, while all samples passed sterility and endotoxin tests. These findings support the consistent production and broader accessibility of FAP-targeted oncological PET imaging services. Therefore, the optimized manual protocol provides a feasible and reliable alternative for nuclear medicine facilities without automated synthesis modules.

INTRODUCTION

Positron Emission Tomography (PET) is an essential functional imaging modality in modern oncology, providing molecular-level biological information that cannot be obtained through conventional anatomical imaging. (Unterrainer et al., 2020) The development of gallium-68 (^{68}Ga) as a PET radionuclide using a ^{68}Ge/^{68}Ga generator system has expanded access to PET radiopharmaceutical production in cyclotron-free facilities (Sullivan & Feinn, 2012), facilitating the translation of research findings into clinical applications (Velikyan, 2015)

Fibroblast Activation Protein (FAP) is a serine protease that is predominantly expressed in cancer-associated fibroblasts (CAFs) across various carcinomas but is minimally expressed in most healthy adult tissues. (Puré & Blomberg, 2018) These highly selective expression characteristics make FAP an attractive target for tumor microenvironment-based imaging and cancer therapy. (Chen & Song, 2019) Among the developed FAPI compounds, FAPI-46 exhibits longer tumor retention and higher image contrast than FAPI-04, making it a promising diagnostic and theranostic candidate (Fouillet et al., 2024).

[^{68}Ga]Ga-DOTA-FAPI-46 has demonstrated superior diagnostic performance in various malignancies, including pancreatic cancer, cholangiocarcinoma, and head-and-neck

tumors, particularly in conditions where [^{18}F]FDG exhibits limitations due to high background metabolic activity.(Kratochwil et al., 2019) Its rapid biodistribution and high tumor-to-background ratio within a short post-injection timeframe contribute to the clinical advantages of this radiopharmaceutical (Giesel et al., 2019).

Positron emission tomography combined with computed tomography (PET/CT) has become an essential component of modern oncology because it provides functional and molecular information that cannot be obtained solely through anatomical imaging. Although [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) remains the most widely used PET radiopharmaceutical, its diagnostic performance may be limited in tumors with low glucose metabolism and in anatomical regions characterized by high physiological glucose uptake. Furthermore, inflammatory and infectious processes may cause nonspecific [^{18}F]FDG accumulation, potentially complicating lesion interpretation. These limitations have encouraged the development of radiopharmaceuticals targeting biological components of the tumor microenvironment rather than tumor-cell glucose metabolism alone. One promising target is fibroblast activation protein (FAP) (Fitzgerald & Weiner, 2020), a membrane-bound serine protease that is highly expressed by cancer-associated fibroblasts within the stroma of many epithelial tumors but has limited expression in most healthy adult tissues (Hamson et al., 2014).

Among available FAP-targeting agents, [^{68}Ga]Ga-DOTA-FAPI-46 has attracted considerable attention because of its rapid biodistribution, high tumor-to-background ratio, and relatively prolonged tumor retention. Recent meta-analytic evidence showed that [^{68}Ga]Ga-FAPI-46 PET/CT achieved higher pooled sensitivity and specificity for cancer diagnosis than [^{18}F]FDG PET/CT, with sensitivity values of 0.96 and 0.73 and specificity values of 0.92 and 0.83, respectively (Abbasi et al., 2025). FAPI PET/CT has also demonstrated promising diagnostic performance in pancreatic, hepatobiliary, gastrointestinal, head-and-neck, and other stromally enriched cancers (Chandekar et al., 2023). For example, comparative evidence in biliary tract cancers showed higher tumor uptake and improved detection of lymph-node and distant metastases with [^{68}Ga]Ga-FAPI PET/CT compared with [^{18}F]FDG PET/CT. These clinical advantages have increased the demand for reliable, reproducible, and accessible production protocols for [^{68}Ga]Ga-DOTA-FAPI-46.

Gallium-68 is particularly relevant for facilities with limited radionuclide production infrastructure because it can be obtained from a germanium-68/gallium-68 (^{68}Ge/^{68}Ga) generator without requiring an on-site cyclotron. The radionuclide has a physical half-life of approximately 68 minutes, which is compatible with the pharmacokinetic characteristics of many small peptides and molecular targeting agents. Nevertheless, its short half-life requires radiolabeling, quality control, and clinical administration to be completed within a limited timeframe. Production efficiency therefore depends strongly on radiochemical yield (RCY), radiochemical purity (RCP), reaction consistency, and preparation time. The International Atomic Energy Agency has emphasized the importance of reliable access to gallium-68 radiopharmaceuticals for cancer diagnosis and theranostic applications (Lepareur, 2022). In facilities without automated synthesis modules, a validated manual procedure may provide a practical alternative, provided that the resulting product meets radiopharmaceutical quality and safety requirements (Da Pieve et al., 2022).

Previous research has established several approaches for [^{68}Ga]Ga-DOTA-FAPI-46 production. (Spreckelmeyer et al., 2020) successfully produced the radiopharmaceutical using

two fully automated synthesis modules, with the optimal process using 50 µg of FAPI-46 precursor and producing a preparation that met relevant requirements for sterility, stability, and radiochemical purity. (Alfteimi et al., 2022) subsequently developed a Good Manufacturing Practice (GMP)-compliant automated procedure without prepurifying the generator eluate. Their process was evaluated using two $^{68}Ge/^{68}Ga$ generator types and three automated synthesis configurations, demonstrating that differences in generators, modules, and synthesis conditions could influence production performance. These studies confirmed the clinical feasibility of automated production but were primarily designed for facilities equipped with commercial synthesis modules and compatible disposable cassettes (Mori et al., 2024).

Further developments have continued to focus predominantly on automation (Davey & Paterson, 2023). Morandeau et al. (2024) developed an automated synthesis procedure using the iPHASE MultiSyn synthesizer, while (Paty et al., 2024) proposed a versatile automated approach that allowed multiple generator elutions and used strong cation-exchange cartridges to trap more than 99.9% of gallium-68 during prepurification. (Rubira et al., 2024) also reported GMP-compliant automated radiolabeling and analytical quality-control procedures for clinical FAP-targeted PET imaging. However, (Meisenheimer et al., 2020) noted that gallium-68 radiopharmaceuticals are still frequently prepared manually despite the potential for increased operator radiation exposure and production variability (Wu et al., 2025). Their comparison demonstrated that process reliability and reproducibility, rather than automation alone, should be considered when selecting a production method.

Despite these advances, important gaps remain in the evidence regarding manual [^{68}Ga]Ga-DOTA-FAPI-46 production. Most published studies have evaluated automated systems, individual synthesis platforms, generator configurations, or prepurification procedures (Brom et al., 2016). The limited studies addressing manual production have generally applied predetermined reaction conditions or modified one factor at a time without statistically evaluating interactions among critical process variables. This limitation is important because gallium-68 complexation is simultaneously influenced by buffer pH and reaction temperature. An unsuitable pH may promote Ga^{3+} hydrolysis and the formation of colloidal gallium species, whereas insufficient temperature may reduce complexation efficiency. Conversely, increasing the temperature under unfavorable pH conditions may accelerate hydrolysis rather than improve chelation. Therefore, separate assessments of pH and temperature may produce incomplete or misleading recommendations for routine radiolabeling procedures.

Addressing this gap is important for nuclear medicine facilities that rely on $^{68}Ge/^{68}Ga$ generators but do not have automated synthesis modules. An optimized manual protocol could reduce failed production batches, improve the usable activity obtained from each generator elution, and increase the number of patient doses prepared without requiring immediate investment in expensive automated equipment. Nevertheless, a high radiochemical yield (RCY) alone is insufficient to establish clinical feasibility. The final preparation must also demonstrate adequate radiochemical purity (RCP), stability during the intended period of use, sterility, and acceptable bacterial endotoxin levels. Therefore, a systematic experimental design evaluating both individual and interactive effects of critical variables is necessary to establish robust operating conditions and determine whether a manual production procedure can consistently meet relevant radiopharmaceutical quality requirements (Montgomery, 2013).

The novelty of this research lies in applying a replicated 3 × 2 full factorial design to the manual radiolabeling of [^{68}Ga]Ga-DOTA-FAPI-46 using a ^{68}Ge/^{68}Ga generator. Unlike one-factor-at-a-time optimization, this design simultaneously evaluated three sodium acetate buffer pH levels (3.7, 4.0, and 4.5) and two reaction temperatures (95°C and 105°C) while estimating their main and interaction effects on RCY. The study also integrated production optimization with assessments of RCP, serum-free and human serum stability, sterility, and bacterial endotoxin levels. This integrated approach provided statistical and pharmaceutical evidence for identifying optimal conditions while determining whether the effect of temperature varied according to the pH environment. Accordingly, this study contributed methodological evidence regarding factorial optimization and practical evidence regarding the feasibility of manual FAPI-46 production.

Therefore, this study aimed to determine the optimal pH and reaction temperature combination for the manual radiolabeling of [^{68}Ga]Ga-DOTA-FAPI-46 using a ^{68}Ge/^{68}Ga generator and to evaluate whether the resulting preparation fulfilled essential radiochemical and microbiological quality requirements. Specifically, it examined the individual and interactive effects of pH and temperature on RCY, assessed RCP and in vitro stability, and verified sterility and bacterial endotoxin compliance. The findings extend knowledge regarding the influence of interacting reaction conditions on gallium-68 chelation efficiency. Methodologically, this study demonstrated the value of factorial design for radiopharmaceutical process optimization. Practically, the resulting protocol may support standardized, efficient, and reproducible [^{68}Ga]Ga-DOTA-FAPI-46 preparation in nuclear medicine facilities with limited automation, thereby helping expand access to FAP-targeted PET imaging for patients with cancer.

METHODS

Ingredients

This study used a ^{68}Ge/^{68}Ga (NRT) generator, DOTA-FAPI-46 precursor (MedChem, 50 μg per batch), sodium acetate trihydrate, glacial acetic acid (Merck), 0.9% NaCl (Braun), ultrapure water (Merck), human serum albumin, ethanol, methanol (Merck, J.T. Baker), ammonium acetate (Merck), HLB cartridges (Waters), vacuum vials (Polatom), Soybean–Casein Digest Medium (TSB), Candida albicans positive control (ATCC 10231), Endosafe™ NexGen-PTS cartridges (Charles River), and LAL Reagent Water (LRW).

Tools

Various tools were used in this study, including a labeling manual module, hot cell (Comecer), dose calibrator (Comecer), heating block, Radio-TLC Scanner (Raytest), ITLC (Molpure), 0.22 μm microfilter, micropipette (Eppendorf), NexGen-PTS Reader (Charles River Laboratories), and a 37°C incubator.

Experimental Design

The study used a full factorial design of 3×2 (*full factorial design*), combining three pH buffer levels (3.7; 4.0; and 4.5) with two reaction temperature levels (95°C and 105°C). Each combination was performed tripled (n=3), resulting in a total of 18 experimental units. Controlled variables included: sodium acetate buffer concentration (1.5 M), number of DOTA-FAPI-46 precursors (50 μg), generator elute volume (5 mL HCl 0.1 N), and heating time (10 min).

Labeling Procedure

The manual module is assembled with a 3-way, three-line valve (line 1: reactor vial + spinal needle; line 2: product vial + 0.22 μm microfilter; line 3: HLB cartridge + 20 mL syringe). The HLB cartridge was activated sequentially with 1 mL of ethanol, 1 mL of ultrapure water, a mixture of HCl 0.1 N:buffer (8:1), and 1 mL of ultrapure water. A total of 0.4 mL of 1.5 M sodium acetate buffer according to the test pH and 50 μg of DOTA-FAPI-46 were fed into the reactor vial, then eluted with 5 mL of HCl 0.1 N from the generator at a rate of ±2 mL/min. After elution, the reactor vial is heated on a *heating block* to the test temperature for 10 minutes without a ventilation needle. After heating, 4 mL of 0.9% NaCl is first added into the reactor vial before the product is purified through the HLB cartridge by pulling the entire liquid in the reactor vial through line 3. The 3-way valve is directed towards the product vial. [^{68}Ga]Ga-DOTA-FAPI-46 captured in an HLB cartridge was then eluted with 1 mL of ethanol, and final formulated with 9 mL of 0.9% NaCl.

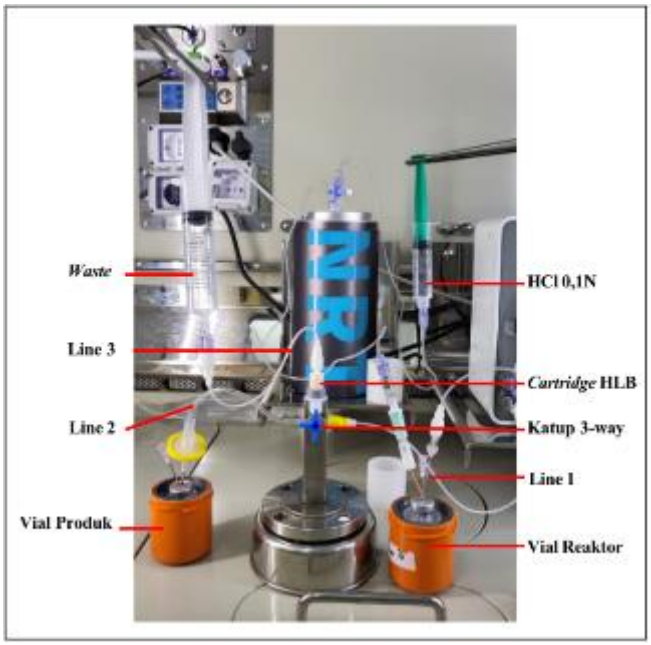

Figure 1. Series of labeling manual modules [^{68}Ga]Ga-DOTA-FAPI-46

Determination of RCY and RCP

RCY is calculated as the percentage of activity [^{68}Ga]Ga-DOTA-FAPI-46 against the total activity of the entire component, after decay correction using: $A_t = A0 \times e^{-\lambda t}$ ($\lambda = \ln 2/68$ minutes). RCP is determined using a Radio-iTLC Scanner with two phases of motion: (1) 0.1 M sodium citrate to determine free Ga^{3+} ; (2) methanol:ammonium acetate 1 M (1:1) to determine colloidal ^{68}Ga.[11]

In Vitro Stability Test

Serum-free stability was evaluated at room temperature for t = 0, 1, and 3 hours. Stability in human serum was achieved by mixing 250 μL serum and 50 μL preparations (5:1 ratio), incubated at 37°C, analyzed at t = 1 and t = 3 h using Radio-TLC.[12] The Gospel of Jesus Christ

Sterility and Endotoxin Tests

The sterility test was carried out by direct inoculation of 0.3 mL of samples to 3 mL of TSB media, incubated for 14 days according to FI VI <71>. Endotoxin assays using NexGen-

PTS with 50× sample dilution in LRW, inserted 25 µL into each *cartridge* well, with a pass limit of <175 EU/dose (<35 EU/mL).

Statistical Analysis

The homogeneity of the variance was verified with Levene's test. RCY was analyzed with bidirectional ANOVA (3×2 design) with *post hoc* Tukey HSD assays. Stability without serum was analyzed by *repeated measures* of ANOVA with Greenhouse-Geisser correction (if the sferity assumption was violated, it was tested with Mauchly's test). Serum stability was analyzed by *paired t-test*. The magnitude of the effects was reported as η^2 (ANOVA) and Cohen's d (t-test). Statistical significance: $\alpha = 0.05$ (SPSS v21).

RESULTS AND DISCUSSION

Radiochemical Yield (RCY)

The RCY values of the 18 samples ranged from 68.00% to 88.04% between labeling conditions. Descriptive statistics per condition are presented in Table 1 and Figure 2.

Table 1. RCY Descriptive Statistics per Labeling Condition (n=3). Optimal conditions are displayed in bold print.

pH Buffer	Temperature (°C)	Mean RCY (%)	SD (%)	Min (%)	Max (%)
3,7	95	85,300	0,700	84,800	86,100
3,7	105	85,430	0,626	84,942	86,135
4,0	95	84,811	2,035	83,333	87,132
4,0	**105**	**87,516**	**0,601**	**86,861**	**88,043**
4,5	95	78,079	0,836	77,119	78,647
4,5	105	71,034	2,650	68,000	72,900

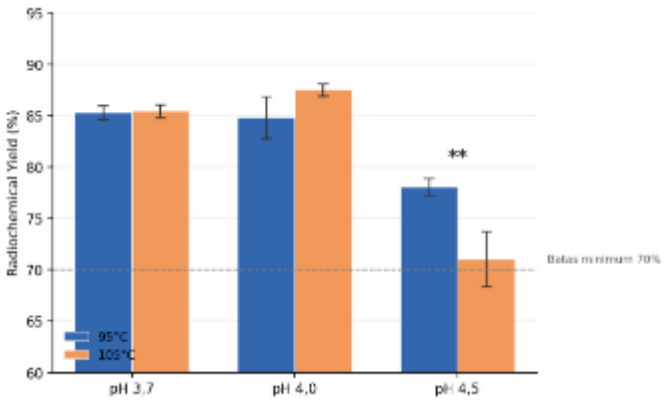

Figure 2. Radiochemical *yield* graph (mean ± SD) per labeling condition

Levene's test showed the homogeneity of the variance was met ($F = 0.573$; $p = 0.720$). The results of the two-way ANOVA are presented in Table 2. pH is the dominant factor with *a very large effect size* ($\eta^2 = 0.951$), while the effect of pH × temperature interaction is also very significant ($\eta^2 = 0.745$). The main effect of temperature independently was not significant ($p = 0.067$).

The post hoc *HSD tukey* showed that pH 3.7 and 4.0 were not significantly different ($p = 0.869$), but they differed significantly from pH 4.5 ($p < 0.001$). Simple *effect analysis*

confirmed that the increase in temperature from 95°C to 105°C had only a significant effect on pH 4.5 (p = 0.001; decrease in RCY 7.045%), while there was no significant effect on pH 3.7 (Δ = +0.130%; p = 1.000) and pH 4.0 (Δ = +2.705%; p = 0.288).

***Radiochemical Purity* (RCP)**

The RCP of all 18 samples at t = 0 ranged from 99.3%–100%, with a mean of 99.876% ± 0.195%. All values exceeded the minimum limit of pharmacopoeia requirements (≥95%). Bidirectional ANOVA for RCP showed a significant effect of pH (F = 9.083; p = 0.004), but there was no effect of temperature (p = 0.266) nor a pH × temperature interaction (p = 0.096). The difference in RCP between pH in absolute terms was only ±0.7%—statistically significant but not clinically significant.

Table 2. Two-Way ANOVA Results for RCY

Source of Variation	SS	df	MS	F	p	h^2
pH	504,39	2	252,20	115,45	<0.001*	0,951
Temperature	8,865	1	8,865	4,058	0,067 (ns)	0,253
pH × Temperature	76,591	2	38,296	17,531	<0.001*	0,745
Error	26,224	12	2,185	—	—	—

* p < 0.001; ns = insignificant.

Stabilitas *In Vitro*

A summary of the stability test results is presented in Table 3; Figure 3; and Figure 4. The decrease in RCP for 3 hours without serum was statistically significant (F = 6.047; p = 0.015, after *Greenhouse-Geisser correction* due to spherical violation W = 0.498; p = 0.004), but only 0.432% absolutely—clinically meaningless because RCP remained >99%. In human serum, RCP decreased from 98.688% ± 1.160% (t = 1 hour) to 96.900% ± 2.049% (t = 3 hours), statistically significant (T = 5.049; p < 0.001; Cohen's d = 1.074) however is not clinically significant as it remains above 95%.

Table 3. In Vitro Stability Test Results [^{68}Ga]Ga-DOTA-FAPI-46.

Conditions	Time Point	Mean RCP (%) ± SD	Statistical Test
Without serum	t = 0 jam	99.883 ± 0.209	*Repeated measures* ANOVA
(room temperature)	t = 1 jam	99.747 ± 0.373	F = 6.047; p = 0.015
	t = 3 jam	99.451 ± 0.563	(*Greenhouse-Geisser*)
In serum	t = 1 hour	98,688 ± 1,160	*Paired t-test*: T = 5.049
(37°C)	t = 3 hours	96,900 ± 2,049	p < 0.001; Cohen's d = 1.074

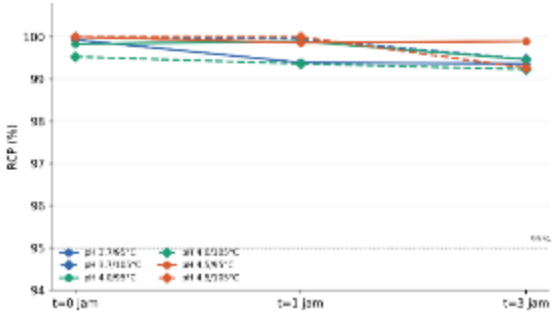

Figure 3. Graph of the stability of serum-free RCP at room temperature (t=0, 1, and 3 h) for six labeling conditions

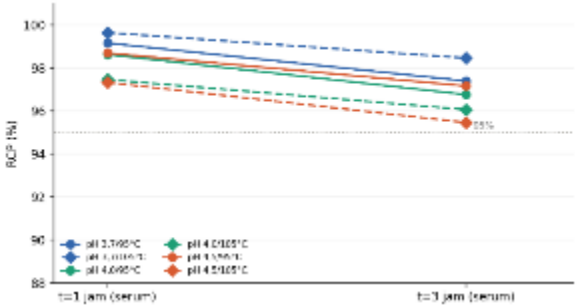

Figure 4. Graph of RCP stability in human serum at 37°C (t=1 and t=3 h) for six labeling conditions.

Sterility and Endotoxin Tests

All 18 samples from the six labeling conditions passed the sterility test after 14 days of incubation, with the negative control remaining clear and the positive control (*C. albicans* ATCC 10231) showing growth on days 3 to 5. All samples also passed endotoxin assays at levels of <35 EU/mL (<175 EU/dose), with all runs valid based on negative control <λ and *spike recovery* in the range of 50–200%.

The main finding of this study is the significance of the effect of pH × temperature interaction ($\eta^2 = 0.745$) on RCY, which confirms that the effect of temperature cannot be evaluated independently of pH in labeling optimization [^{68}Ga]Ga-DOTA-FAPI-46. The pH condition of 4.0/105°C resulted in the highest RCY (87.516% ± 0.601%), consistent with the report of Paty et al. which used an automatic system of pH acetate buffer pH 4/105°C and produced an RCY of >88%.

The identification of these significant interaction effects is an important methodological contribution of this study. The *one-factor-at-a-time* (OFAT) approach that is still commonly used in radiopharmaceutical labeling optimization is inherently inherently incapable of detecting the effects of this kind of interaction, resulting in suboptimal condition recommendations. As an illustration, if temperature is evaluated separately without considering pH, the results at pH 3.7 ($\Delta = +0.130\%$) and pH 4.5 ($\Delta = -7.045\%$) will negate each other, and the resulting conclusions will depend solely on each other. at an arbitrarily selected pH as the "standard condition" of a methodological artifact that can be completely avoided through a full factorial design.

The interaction effects found from the perspective of coordination chemistry, reflect the phenomenon *of competing equilibria* between the Ga^{3+}-DOTA complexation reaction and the Ga 3+ hydrolysis reaction. Both of these reactions are accelerated by temperature rise, but with different pH dependencies: the complexation rate increases optimally at pH 3.7–4.0 where free Ga^{3+} is maximally available, while the hydrolysis rate increases drastically at pH ≥4.5 as the temperature rises due to a shift in speciation equilibrium towards insoluble hydroxide species.

Ga3+ ions at pH 3.7 and 4.0 are in a favorable speciation equilibrium for complexation with the DOTA-FAPI-46 kelator. The DOTA carboxylate group has been deprotonated enough to coordinate with Ga^{3+}, while the risk of colloidal $Ga(OH)_3$ formation is still low. Under this condition, the increase in temperature to 105°C increases the *water exchange rate* synergistically with the availability of Ga^{3+} optimal free of charge, maximizing ligand substitution efficiency.[21] The absence of significant differences between pH 3.7 and pH 4.0 across all temperature combinations (Tukey HSD: $p = 0.869$) suggests that in this pH range the operator has considerable condition flexibility without the risk of significant RCY degradation.

In contrast, a significant decrease in RCY at pH 4.5/105°C (71.034%; $\Delta = -7.045\%$ vs pH 4.5/95°C; $p = 0.001$) can be explained by accelerated hydrolysis of Ga^{3+}. At pH ≥4, the speciation equilibrium shifts towards colloidal $[Ga(OH)]^{2+}$, $[Ga(OH)_2]^{+}$, and $Ga(OH)_3$ that are not reactive to chelation, and this rate of hydrolysis increases significantly as the temperature increases.[9] This is consistent with Burke and Archibald's (2021) theoretical explanation of the acceleration of $Ga(OH)_3$ precipitation at pH 4–5 as temperature rises, and is the first statistical confirmation of this phenomenon in the context of manual labeling of FAPI-46. The high variability at pH conditions of 4.5/105°C (SD = 2.650%) compared to other conditions (SD ≤ 2.035%) also indicates a process instability that is clinically unacceptable for routine

production—not only in terms of RCY averages, but also in terms of reproducibility between *batches*.

Relevance to Generator Variability

It should be noted that the RCY obtained in this study is also influenced by the characteristics of the $^{68Ge/68Ga}$ generator elurate.The presence of metal ion contaminants such as Zn^{2+} 68Ga ^{decay} products accumulated in the generator column can compete with Ga^{3+} in the complexation process and affect RCY.[2] At the time of secular equilibrium is reached, the amount of Zn^{2+} can exceed ^{68Ga} by more than 10 times; therefore, routine dilution prior to synthesis is an important practice for lowering $Zn^{2+\ concentrations}$ up to 5.5 times.[2] This study used a fixed elution volume (5 mL HCl 0.1 N) as a standardization effort, but follow-up studies explicitly evaluating the influence of generator age on RCY in the context of manual methods are still needed to comprehensively quantify this source of variability.

Comparison with Previous Labeling Studies

Meyer et al. (2020) reported manual labeling of [^{68}Ga]Ga-DOTA-FAPI-46 using a 2.5 M sodium acetate buffer at pH 3.3–3.6 and a temperature of 95°C for 20 minutes, but did not explicitly report RCY data.[8] The absence of quantitative RCY data in the study is one of the justifications for this study, which systematically quantifies the effects of pH and temperature variations with adequate replication. (Alfteimi et al., 2022) automated study comparing three different synthesis modules reported RCY ranging from 68–92% depending on the module and the conditions used[24], suggesting that the RCY range found in this study (71–88%) was within an acceptable range and not inherently inferior to automated systems.

(Boonkawin & Chotipanich, 2021) reported that RCY >70% used an iQS-TS automated system with a 0.25 M sodium acetate buffer at 95°C—a value comparable to the suboptimal conditions (pH 4.5/95°C) in this study, confirming that optimization of labeling conditions, rather than mere process automation, is a critical determinant of product quality. These findings have important implications for facilities that are considering investing in automated modules: upgrading from pre-optimized manual methods to automated systems may result in smaller-than-expected RCY improvements, while cost investments can be significant.

Radiochemical Purity and Purification Effectiveness of HLB

The high average RCP of 99.876% across all conditions, with no clinically significant effects of pH or temperature variations, confirmed the consistent effectiveness of HLB cartridge purification. [^{68Ga}]The relatively lipophilic Ga-DOTA-FAPI-46 is retained in the HLB silent phase, while the hydrophilic $^{Ga\text{-}3+}$, Zn^{2+}, $^{68Ge,\ buffer,\ and\ HCl\ pass}$ through the cartridge and are accommodated in the waste.[14] Use of two phases of motion on Radio-iTLC analysis of 0.1 M sodium citrate to determine free Ga^{3+} with Rf values [$^{68Ga]\ Ga\text{-}DOTA\text{-}FAPI\text{-}46\ and\ colloidal\ 68Ga}$ = 0.0–0.3 and free Rf Ga3+ = 0.8–1.0 (Figure 5), and methanol: ammonium acetate 1 M (1:1) to determine colloidal ^{68Ga} with Rf values [$^{68Ga]\ Ga\text{-}DOTA\text{-}FAPI\text{-}46\ and\ Ga3+}$ free = 0.8–1.0 and colloidal ^{68Ga} Rf = 0.0–0.2 (Figure 6) allow the identification and quantification of the two main impurity types separately, resulting in a more comprehensive RCP profile than the use of a single motion phase.[11] Although there was a statistically significant effect of pH on RCP ($F = 9.083$; $p = 0.004$), the absolute difference between groups was only ±0.7% well below the clinically mean threshold confirming that the HLB purification step was able to compensate for the variation in free ^{68Ga} levels produced under different labeling conditions.

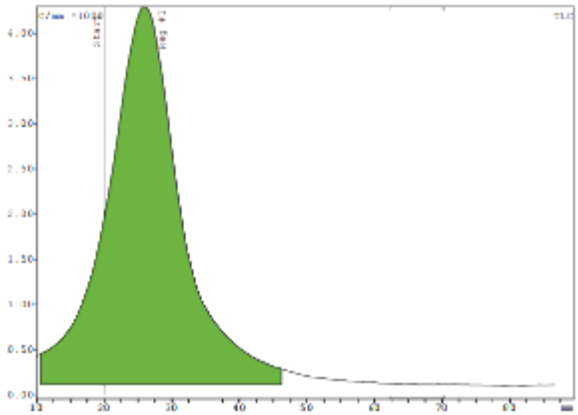

Figure 5. iTLC Scanner Radio Chromatogram in the motion phase of sodium citrate 0.1 M

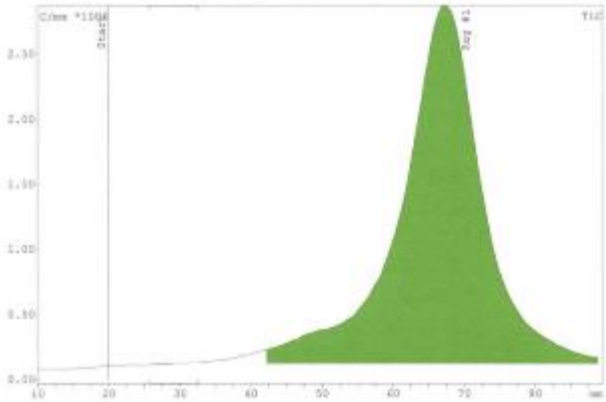

Figure 6. iTLC Scanner Radio Chromatogram in the motion phase Methanol:Ammonium Acetate 1 M (1:1)

Stabilitas In Vitro

The high stability of the [^{68}Ga]Ga-DOTA-FAPI-46 complex for 3 hours at room temperature (RCP 99.451%) reflects the inert kinetic properties of the Ga^{3+}-DOTA complex, where Ga3+ is bound by four nitrogen atoms and two carboxylic oxygenates of the macrocyclic kelator forming an octahedral coordination complex that is highly resistant to dissociation at room temperature.[This 12] 3-hour stability provides essential operational flexibility for facilities serving more than one patient per production cycle, given that the time between production and injection may vary depending on the examination schedule.

The decrease in RCP in human serum from t = 1 h to t = 3 h by 1.788% reflects thermodynamic competition between DOTA kelators and plasma proteins especially albumin and transferrin in binding to Ga^{3+} via a transcleation mechanism. Transferrin is specifically known to have two metal binding sites with a high affinity to Ga^{3+}, but the stability constant of Ga^{3+}-DOTA (log K > 21) exceeds the affinity of Ga^{3+} to transferrin (log K ≈ 20.3), so that the DOTA kelator remains thermodynamically superior in the competition.[12] Although the decrease in RCP in serum was statistically significant (*paired t-test*: T = 5.049; p < 0.001; Cohen's d = 1.074), value at t = 3 hours (96.900% ± 2.049%) remained above the clinical limit of 95%. It is important to distinguish statistical significance from clinical significance: a small p-value at n = 18 is not automatically clinically meaningful if the absolute difference does not exceed the practically relevant threshold.[15] In a clinical context, [^{68}Ga]Ga-DOTA-FAPI-46 is generally injected within 1–2 hours of production, so that the stability value at t = 1 h in serum (98,688%) well above the 95% limit is the most practically relevant parameter.

Sterility and Endotoxin Tests

Sterility test passes on all 18 samples confirmed the effectiveness of the consistent aseptic technique within the *hot cell* and final filtration through a 0.22 μm membrane as the final sterilization barrier. Sterility testing is performed retrospectively in accordance with international regulations that allow *retrospective sterility testing* for short-lived radiopharmaceuticals, as the half-life of ^{68}Ga (67.7 minutes) does not allow for the completion of incubation 14 days before the product is administered to the patient. The validity of this approach depends on the implementation of *a parametric release* system supported by well-documented historical data of the process, including validation of the filtration sterilization method used.

Passing the endotoxin test on the entire sample confirms that the raw materials, solvents, equipment are pyrogen-free, as well as the production environment within *the hot cell* free from Gram-negative bacterial lipopolysaccharide contamination. Validation of each *endotoxin test run through negative control requirements (<λ) and* spike recovery *in the range of 50–200% ensures that no inhibition or activation effect of the sample matrix component on the LAL reagent can result in* false negative *or* false positive *results.*

Clinical Implications and Feasibility of Implementation

Fulfillment of all quality parameters—RCP, *in vitro* stability, sterility, and endotoxin—together confirmed the *clinical readiness* of the manual labeling protocol [^{68}Ga]Ga-DOTA-FAPI-46 at optimal conditions (pH 4.0/105°C). An RCY value of 87.516% under optimal conditions has direct implications for patient service capacity: the higher the RCY, the more doses can be prepared from a single cycle of generator elution, resulting in increased production efficiency without additional generator costs.

It should be noted that manual methods still carry a higher risk of radiation exposure for production personnel than automatic methods, so the implementation of strict radiation protection protocols and adequate use of *hot cells* is an absolute requirement. A study by Meisenheimer et al. (2020) that compared personnel exposure doses between manual and automated methods reported that manual methods resulted in significantly higher finger doses, although they remained below the established annual dose limit. As a follow-up, a quantitative dose comparison study of personnel radiation exposure in the context of this optimized manual labeling protocol needs to be conducted to complete the implementation safety profile.

CONCLUSION

This study demonstrated that the optimal condition for manual [^{68}Ga]Ga-DOTA-FAPI-46 radiolabeling using a ^{68}Ge/^{68}Ga generator was a 1.5 M sodium acetate buffer at pH 4.0 and a reaction temperature of 105°C for 10 minutes. This condition produced the highest radiochemical yield of 87.516% ± 0.601%, while all experimental conditions achieved radiochemical purity above the pharmacopoeial threshold of 95%. Buffer pH significantly affected radiochemical yield, whereas temperature alone did not show a significant effect; however, the significant pH–temperature interaction indicated that these parameters required simultaneous optimization. The preparation remained stable for three hours at room temperature and in human serum, with radiochemical purity maintained above 95%. All samples also met sterility and bacterial endotoxin requirements. These findings supported the

feasibility of the optimized manual protocol as a practical alternative for nuclear medicine facilities without automated synthesis modules.

Future research should validate the optimized protocol through a larger number of production batches and under routine clinical conditions to establish its reproducibility, robustness, and compliance with Good Manufacturing Practice (GMP) requirements. Further studies should evaluate additional variables, including precursor concentration, buffer volume and concentration, generator age, metallic impurities, elution profile, heating duration, and purification procedures. Direct comparisons between manual and automated synthesis methods should also assess radiochemical yield, production cost, processing time, batch consistency, and occupational radiation exposure. Moreover, extended stability testing, analytical validation using high-performance liquid chromatography (HPLC), and preclinical or clinical biodistribution studies are recommended to confirm the preparation's safety, diagnostic performance, and suitability for routine FAP-targeted PET imaging.

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