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Research article

The first radiolabeled ^{68}Ga-FAPI-46 for clinical PET applications using a fully automated iQS-TS synthesis system in Thailand

Natphimol Boonkawin*, Chanisa Chotipanich

National Cyclotron and PET Centre, Chulabhorn Hospital, Bangkok, Thailand

*Corresponding author Email: natphimol.boo@pccms.ac.th

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Abstract

Background: Gallium-68 labeling of various peptides is now widely used in routine clinical positron emission tomography (PET) applications. We used an automated synthesis system at the National Cyclotron and PET Center of Chulabhorn Hospital to reduce human errors, lower the radiation dosages to operators during synthesis, and establish high product yields. **Methods:** In this study, we synthesized ^{68}Ga-labeled FAPI-46 peptide using the fully automated iQS-TS theranostics module. The radiosynthesis of ^{68}Ga-Fapi-46 was completed in 15 min, including generator elution and dispensing radiopharmaceuticals. **Results:** The ^{68}Ga-FAPI-46 radiosynthesis yielded product with 70% ± 2% activity and >95% radiochemical purity. All quality control parameters conformed with the limits prescribed by the European Pharmacopoeia. **Conclusion:** This study demonstrated successful synthesis of ^{68}Ga-labeled FAPI-46 peptide using the fully automated iQS-TS theranostics module, which produced reliable and reproducible synthetic yields. The ^{68}Ga-FAPI-46 product is of high purity that exceeds minimum recommended requirements.

Keywords: ^{68}Ga-Fapi-46, Automated synthesis, Fibroblast activation protein (FAP)

Introduction

Fibroblast activation protein (FAP) is a human enzyme encoded by the *FAP* gene that shows increased expression in the stroma of a variety of malignancies, including breast, colon, and pancreatic carcinomas. FAP expression is also observed in more than 90% of activated stromal fibroblasts in all human carcinomas.[1,2] Recently, several new radiopharmaceuticals have been designed and tested at preclinical phases. Quinoline-based FAP is a highly promising molecular-targeted imaging probe that is also a specific inhibitor, and thus hopefully has therapeutic utility. Several FAP inhibitor (FAPI) series have been developed for clinical diagnostic and therapeutic applications, such as FAPI-01, FAPI-02, FAPI-04, and FAPI-21; the evolution of FAPI variants is shown in Figure 1.

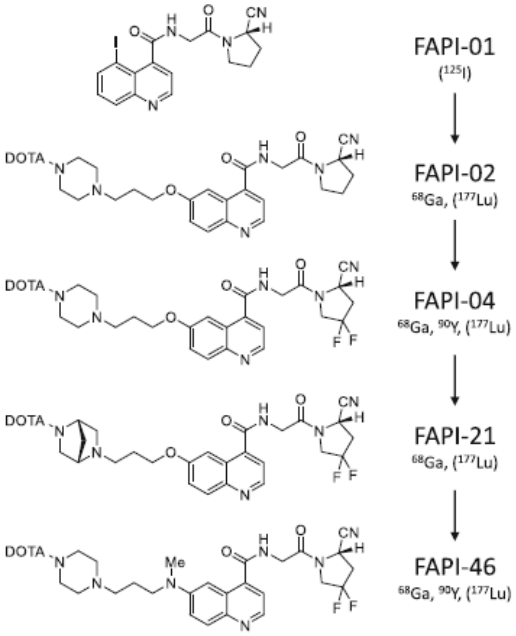


Figure 1. Examples of FAP-targeted theranostics used in preclinical experiments with nuclides in brackets.[3]

^{68}Ga conjugated with DOTA-based derivatives has rapidly gained interest in the field of PET radiopharmacology, with new agents being recently developed, such as ^{68}Ga-PSMA-11 and ^{68}Ga-Dotatate. However, radiopharmaceuticals are mostly produced by manual synthesis. Currently, automated systems are more advantageous than manual methods in terms of radiation protection and compliance with good manufacturing practice (GMP). Also, there is more equipment required for hospital-based radiopharmaceuticals.[4,5]

In this study, we describe the procedure and quality control for labeling FAPI-46 peptide using the fully automated iQS-TS theranostics synthesizer supplied by Isotope Technologies Garching GmBH ITG (Munich, Germany).

2. Materials

The iQS-TS fully automated theranostics synthesizer supplied by Isotope Technologies Garching GmBH ITG was used to produce FAPI-46 peptide labeled with ^{68}Ga. The ^{68}Ge/^{68}Ga generator and labeling kits were also supplied by Isotope Technologies Garching GmBH ITG. The kits consisted of Hydrochloric acid (0.05 M) as the eluent for the generator, sodium acetate (0.25 M), ethanol, NaCl, a C18 SPE Sep-Pak cartridge, 15 mL Sterile vials, vent filters, and 0.22-µm pore size Cathivex filters. All chemicals in the kit were of pharmaceutical grade.

The patented FAPI-peptide product was provided from Sofiei Theranostics (Dulles, VA, USA) as a 1 mg powder, which was dissolved in 2 mL H_2O and used in 100-µL aliquots. The reagents used for instant thin-layer chromatography (ITLC) and high pressure liquid chromatography (HPLC) to determination the radiochemical purity (RCP) of the product were HPLC grade. The trifluoroacetic acid (HPLC grade, >99.90% purity) and acetonitrile (CHROMASOLV®, HPLC grade, >99.90% purity) were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade water, ammonium acetate, and methanol (CHROMASOLV®, HPLC grade, >99.90% purity) were supplied from RCI Labscan (Bangkok, Thailand).

3. Methods

3.1 Generator

On non-synthesis days, the generators were eluted within 24 h before FAPI synthesis due to the build-up of metal ions on the column. The elution process involved using an elution cassette and 0.05 M HCl solution driven by the ITG system in approximately 10 min. The elution volume for radiopharmaceutical synthesis was 12 mL. The generator eluent was direct to the reactor.

3.2 Automated radiolabeling process

The iQS-TS was performed as an automated pre-run machine test to guarantee functionality before synthesis. Then, a sterile synthesis cassette was removed from its packaging. All valves were connected and tightened before putting the cassette into synthesizer. The preparation and connection of reagents were performed with appropriate tubing to the reactor vessel with 50 µg of DOTA-FAPI peptide in 1.0 mL sodium acetate buffer (0.25 M) solution. The process is shown in Figure 2.

The radiolabeling process was divided into three steps. First, the generator was eluted from the $^{68}Ge/^{68}Ga$ radionuclide generator to the reaction vessel using 4 mL of HCl (0.05 M). The second step was labeling process, which involved pre-heating the vessel to 95°C, with continued heating for 10 min after mixing the peptide with $GaCl_3$. The last step was purification, which involved loading the mixture into a C18 SPE Sep-Pak cartridge (pre-activated using 1.0 mL of ethanol solution, followed by 4 mL of 0.9% NaCl solution) to trap labeled peptide.

The SPE cartridge was subsequently washed with 5 mL of 0.9% NaCl in the waste bottle. The final product was eluted from the C18 cartridge by flashing with 0.5 mL of ethanol, and then transferred through the 0.22-µm Cathivex-GV filter (ABX, Germany) into the product vial, before being further diluted with 9.5 mL of 0.9% NaCl. The 0.22-µm filter and needles were removed from the product vial, then put into a waste bottle by the operator. Testing of the 0.22-µm filters was automated by the theranostics software as the filter integrity test. The dynamic graphic synthesis report was saved on the computer.

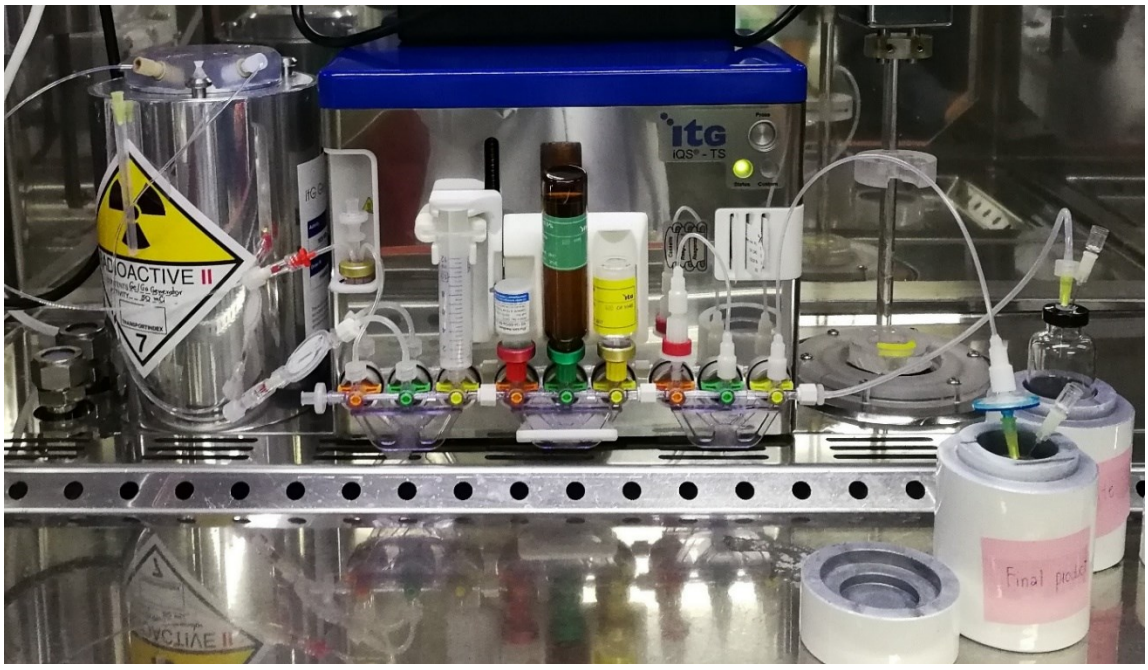


Figure 2. Set up of the fully automated iQS-TS theranostics synthesizer.

3.3 Quality control

Quality control methods were based on the European Pharmacopoeia monograph.[6] The labeled solution was visually inspected for being clear, colorless, and within a pH range of 4.0–8.0. The identity of the final product was confirmed by analytical HPLC and compared with the reference standard (Sofie iTheranostics). Confirmation of radiochemical purity was conducted by ITLC and HPLC. Half-life determination and the gamma spectrum of the main peak were used to confirm the identity of the radionuclide. The ^{68}Ge content was determined by the 48-h decay of ^{68}Ga in a calibrated multichannel analyzer (CANBERRA). Residual solvents were determined by gas chromatography. The filter integrity test, endotoxin measurements, and sterility tests were also performed.

ITLC analyses of ^{68}Ga-FAPI-46 were performed using an AR-2000 radio scanner (Bioscan, Washington DC, USA). A mixture of 75% methanol and 25% 5 M ammonium on ITLC-SG paper (Merck, Kenilworth, NJ) was used to determine the percentage ^{68}Ga-FAPI-46 and ^{68}Ga impurities.

The percentage of ^{68}Ga-FAPI-46 in the final product was determined by analytical HPLC, using a system equipped with a C18 column (5µm × 4.6 mm × 150 mm, Atlantis™T3, Waters, MA, USA), a UV detector operating at 264 nm, and a radio-detector (Bioscan B-FC 3200, Eckert & Ziegler Radiopharma, Wilmington, MA, USA). The eluent mixture of HPLC water (A) and acetonitrile (B) were both supplemented with 0.1% (v/v) trifluoroacetic acid at a flow rate of 1.5 mL/min and 25°C as follows: (t= 0) 13.0% B, (t=12.50) 13% B, (t=12.51) 100% B, (t=17) 100% B, (t=17.1) 13% B, and (t=20) 13% B. The final product was confirmed by co-elution with the non-radiolabeled reference standard FAPI-46 (Figure 3). Finally, the stability of ^{68}Ga-Fapi-46 was obtained.

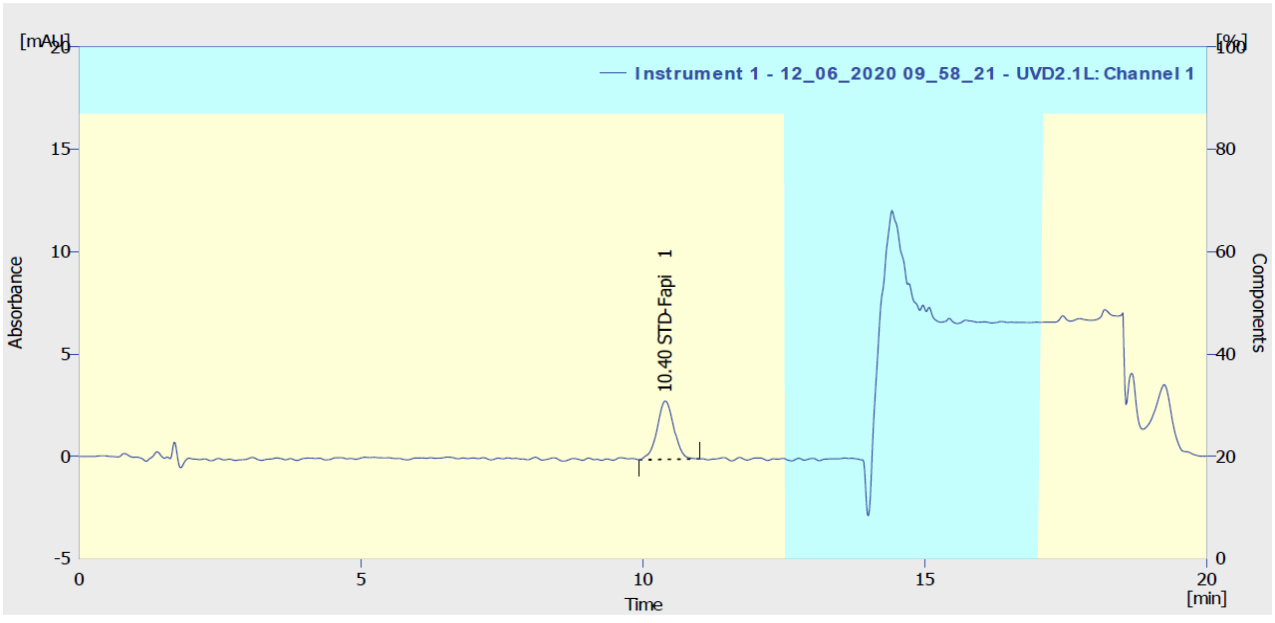


Figure 3. UV chromatogram of the non-radiolabeled FAPI-46 reference standard solution (10 µg/mL).

4. Results and Discussion

The automated synthesis of ^{68}Ga-FAPI-46 was achieved with a radiochemical purity >95% . The yield percentage was >70%. The activity of the ^{68}Ga-labeled FAPI peptide was approximately 23–25 mCi from the 40 mCi eluted by the ITG automated synthesis module. The volume of the product was approximately 10 mL. Total time of synthesis was approximately 15 min. The steps of synthesis are shown in Figure 4.

The ^{68}Ga-labeled FAPI peptide product solution was clear, colorless, particle-free, and sterile. The pH of the product was in the range of 4.0–8.0. The final products contained <10% residual ethanol. The level of endotoxins was <14. 58 EU/ mL. Finally, the ^{68}Ge content in the final ^{68}Ga-FAPI peptide products did not exceed 0.005% of the total activity.

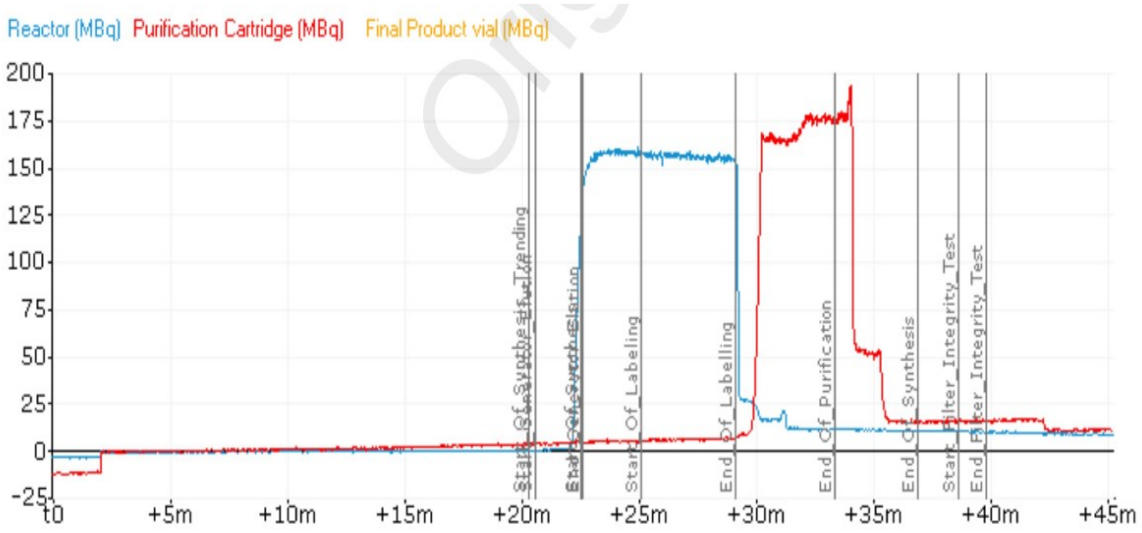


Figure 4. Graphical presentation of the automated synthesis steps of ^{68}Ga-FAPI-46

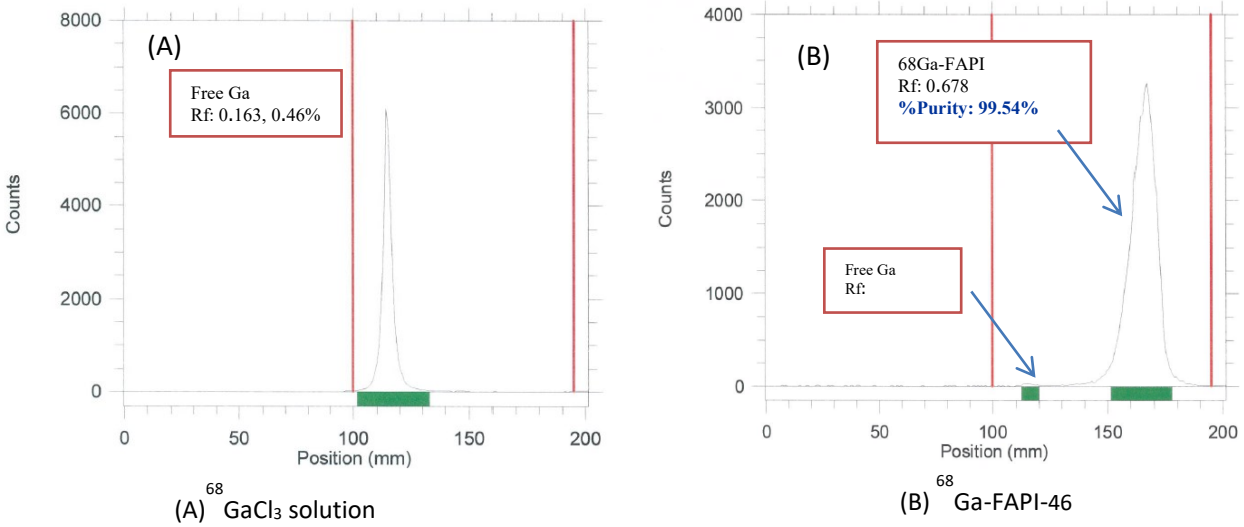
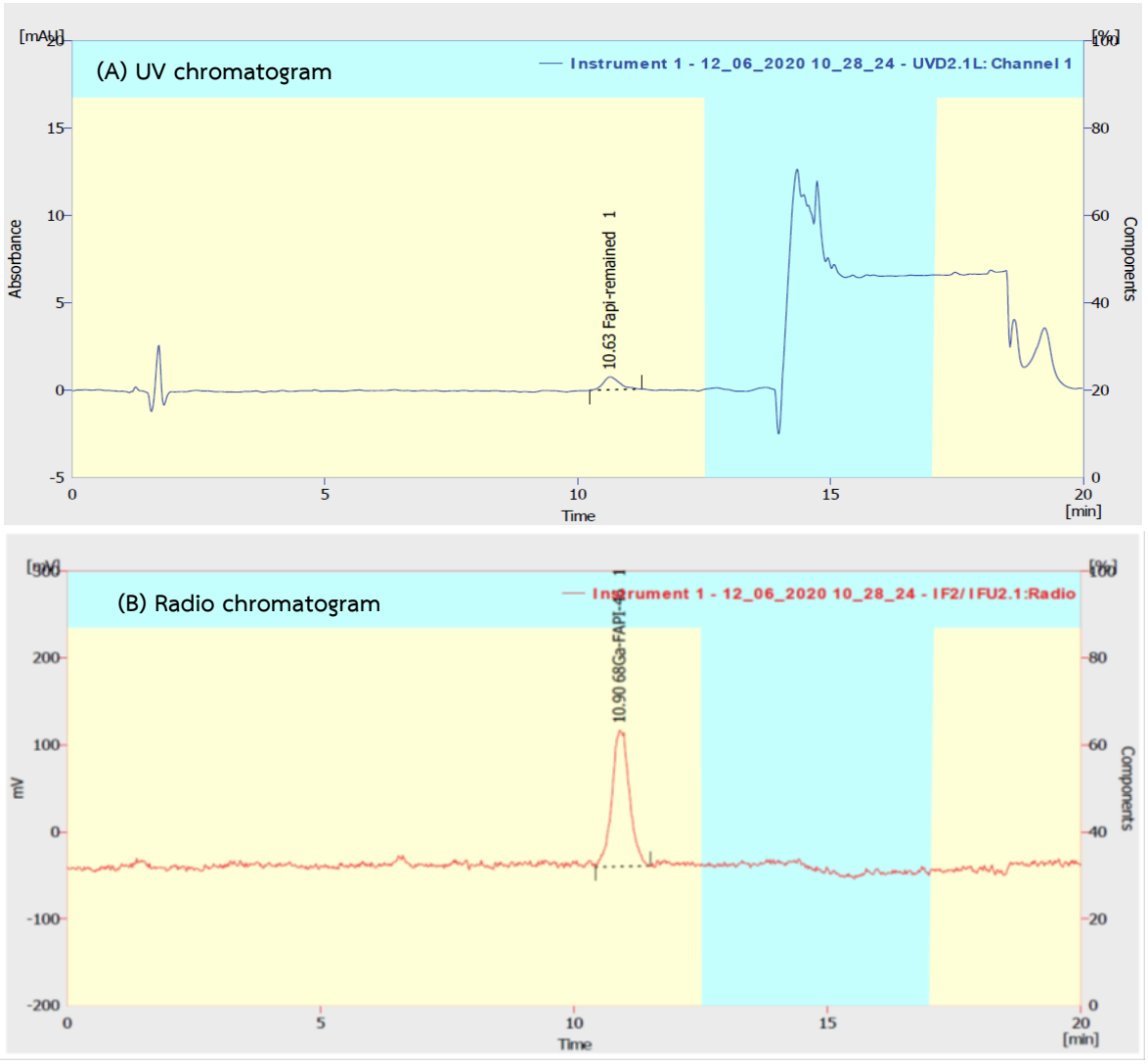


Figure 5. ITLC chromatograms on SG paper in 75:25 Methanol:5 M Ammonium Acetate (A) and ^{68}GaCl$_3$ solution, (B) ^{68} Ga-FAPI-46.

Radiochemical purity was determined by instant thin-layer chromatography on silica gel impregnated glass fibers (ITLC-SG) and the results are shown in Figure 5A and 5B. The free-Ga peak appeared at R_f between 0.1 and 0.2. The ^{68}Ga-FAPI-46 peak was observed at R_f between 0.6 and 0.8. The percentage of ^{68}Ga-Fapi-46 was >95%.

The results of the radiochemical and chemical purity analysis of ^{68}Ga-FAPI-46 using reverse-phase HPLC demonstrated that the radiochemical purity of ^{68}Ga-FAPI-46 was >95% , which could be integrated by the area of the radio peak (Figure 6A) . The 50% mixture product sample and 50% reference solution were measured to confirm the identity of the ^{68}Ga-FAPI-46 product (Figure 7).



Result Table (Uncal - Instrument 1 - 12_06_2020 10_28_24 - IF2/IFU2.1:Radio)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	10.900	3781.538	156.720	100.0	100.0	0.38	68Ga-FAPI-46
	Total	3781.538	156.720	100.0	100.0		

Figure 6. HPLC chromatograms of ^{68}Ga-FAPI-46 product solutions: (A) UV chromatogram, (B) radio chromatogram

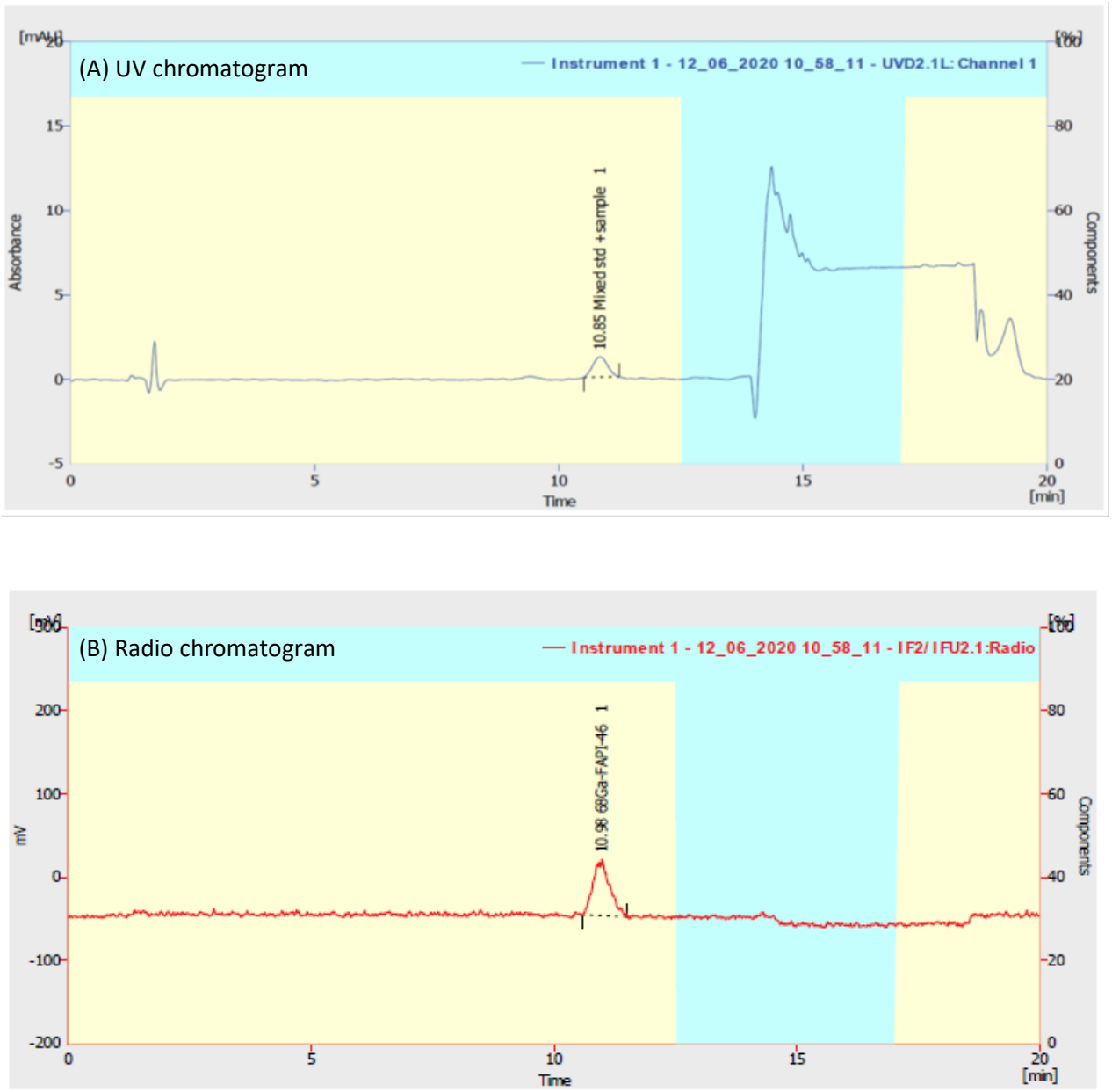


Figure 7. HPLC chromatogram of co-injecting the ^{68}Ga-FAPI-46 product and reference standard: (A) UV chromatogram, (B) radio chromatogram.

Stability of the labeled product was studied over a 4 h period after production using ITLC and HPLC chromatography. The stable product with a radiochemical purity of >95% over the period of study is shown in Table 1. However, in this study, the product was used within 60 min of preparation.

Table 1. The radiochemical purity and stability of ^{68}Ga-FAPI-46 4 h after production

Time point	ITLC		HPLC	
	^{68}Ga-FAPI-46 (%)	Free ^{68}Ga (%)	^{68}Ga-FAPI-46 (%)	Free ^{68}Ga (%)
T0	99.54	0.46	100	0
T1	99.49	0.51	100	0
T2	98.84	1.16	100	0
T3	99.50	0.50	100	0
T4	99.14	0.86	100	0

5. Conclusion

Synthesis of ^{68}Ga-peptide with an automated synthesis system is reliable, reproducible, and ensures consistent product yields. The iQS-TS fully automated theranostics synthesizer is an innovative system to reliably perform radiosynthesis with a relatively low incidence of failures. The ^{68}Ga-FAPI-46 product was of high purity that exceeds minimum recommended requirements. This system is GMP-compliant with regard to safety and can reduce radioactivity exposure to the operator.

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(https://www.edanz.com/ac)

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