Optimization of Labeling PSMA^{HBED} with Ethanol-Postprocessed ^{68}Ga and Its Quality Control Systems

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Radiolabeling of the prostate-specific membrane antigen (PSMA) inhibitor Glu-NH-CO-NH-Lys(Ahx) using the ^{68}Ga chelator HBED-CC (PSMA^{HBED}) allows imaging of prostate cancer lesions because of high expression of PSMA in prostate carcinoma cells and in bone metastases and lymph nodes related to the disease. The aim of this work was to optimize labeling of ^{68}Ga-PSMA^{HBED} using the efficient cation-exchange postprocessing of ^{68}Ga as well as the development of a thin-layer chromatography (TLC)–based quality control system. **Methods:** Labeling was optimized for online ethanol-postprocessed ^{68}Ga eluate investigating various parameters, such as buffer molarity (0.1–1 M), temperature (25°C–90°C), tracer amount (0.11–0.74 nmol), and labeling time. In addition, purification of the crude product was tested. For radio-TLC quality control, various mobile phases were analyzed using silica gel 60 plates and the results were validated using high-performance liquid chromatography. The most superior mobile phases were also applied on instant thin-layer chromatography (ITLC) silica gel plates. **Results:** Using optimized conditions, labeling yields of more than 95% were obtained within 10 min when ethanol-based postprocessing was applied using PSMA^{HBED} amounts as low as 0.1 nmol. A higher precursor concentration (0.7 nmol) further increased labeling and quantitative yields to more than 98% within 5 min. In clinical routine, patient batches (>200 applications) with radiochemical purity greater than 98% and specific activities of 326 ± 20 MBq/nmol are obtained reproducibly. When TLC quality control was performed on silica gel 60 plates, 4 mobile phases with suitable separation properties and complementary R_f values were identified. Two systems showed equivalent separation on ITLC silica gel plates, with ITLC analysis finished within 5 min, in contrast to 20 min for the TLC system. Labeling of PSMA^{HBED} was optimized for cation-exchange postprocessing methods, ensuring almost quantitative labeling and high nuclide purity of final ^{68}Ga-PSMA^{HBED}, making subsequent purification steps unnecessary. **Conclusion:** The new radio-TLC method allows quality control in a short time using a fast, reliable, low-cost method with little equipment complexity. Using this approach, the synthesis is easily adopted by automated synthesis modules.

Key Words: ^{68}Ga; PSMA; generator postprocessing; quality control; ITLC

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Prostate-specific membrane antigen (PSMA) is a cell surface protein with increased expression on nearly all prostate cancer cells compared with other PSMA-expressing tissues such as kidney, proximal small intestine, or salivary gland (*1–3*). Because PSMA expression is restricted to the prostate and the cell surface at all stages of disease, it holds promise as a target for specific imaging and therapy of prostate cancer and neovasculature (*4–6*). Studies have recently shown that low-molecular-weight peptidomimetic radiopharmaceuticals are clinically attractive because prostate cancer lesions can be imaged with high contrast and higher sensitivity than is possible with ^{18}F-choline-PET/CT (*7–10*). Noninvasive imaging of increased PSMA expression provides important information on the stage of prostate cancer and the location of metastatic lesions.

One of those peptidomimetic radiopharmaceuticals is Glu-NH-CO-NH-Lys(Ahx)-HBED-CC (PSMA^{HBED}, PSMA-11), showing high potential as a prostate cancer imaging agent (*1,8*). It is a urea-based PSMA inhibitor including the acyclic complex ligand *N,N′*-bis[2-hydroxy-5-(carboxyethyl)benzyl]ethylenediamine-*N, N′*-diacetic acid (HBED-CC), being recently proposed as a chelator for efficient radiolabeling with generator-produced ^{68}Ga at room temperature (*11*). In addition, the lipophilic character of the ^{68}Ga complex of HBED-CC was found to be a necessary feature for interaction with the PSMA binding site (*1,12,13*).

Generator-produced ^{68}Ga represents an attractive alternative to cyclotron-based PET nuclides such as ^{18}F or ^{11}C but requires protocols to provide ^{68}Ga suitable for medical use. Several methods have been developed for purification of ^{68}Ga eluate to fulfill regulatory requirements (*13–15*). Initial publications on ^{68}Ga-PSMA^{HBED} used crude ^{68}Ga generator eluate for ^{68}Ga labeling of PSMA and high-performance liquid chromatography (HPLC) for quality control. This report describes radiolabeling of PSMA^{HBED} using cation-exchange–based postprocessing methods for manual synthesis as well as the use of an automated module followed by the development of a thin-layer chromatography (TLC)– and instant TLC (ITLC)–based quality control system.

MATERIALS AND METHODS

Only the highest-reagent-grade chemicals and TraceSELECT water were used. Chemicals were purchased from Sigma Aldrich and used without further purification, unless stated otherwise. ^{68}Ga was obtained from an initially 1.1-GBq ^{68}Ge/^{68}Ga generator (2 y old) from Cyclotron Co. Ltd. and from an initially 1.85-GBq ^{68}Ge/^{68}Ga generator (new) from iThemba Labs.

BioRad AG 50W-X4 (200–400 mesh) cation-exchange resin was used to prepare a microchromatography column (50 mg of resin, 2-mm inner diameter, 5-mm length). Also, Varian Bond Elut-SCX

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was used. Labeling reactions were performed in 11-mL glass vials (Mallinckrodt) using a block thermostat (TK13; Ditabis) for temperature control and agitation. Purification was performed with 30-mg C-18 cartridges (Phenomenex Strata-X Tubes). Activity was measured using a curie meter (ISOMED 2010; Nuklear-Medizintechnik Dresden GmbH). pH was measured using a calibrated pH meter (SevenEasy pH; Mettler-Toledo). TLC plates (aluminum-backed silica gel 60; Merck) and ITLC silica gel plates (Varian) were analyzed using a flat-bed scanner (Instant Imager [Canberra Packard] or miniGita [Raytest-Isotopenmessgeräte GmbH]).

Reverse-phase HPLC using a LiChrosphere 100-RP18EC column (5 mm, 250 × 4 mm) was applied to quantify the radiochemical purity of ^{68}Ga-PSMA. HPLC was equipped with a Hitachi L-7100 pump coupled with ultraviolet (Hitachi L-7400) and radiometric (γ-Raytest-Isotopenmessgeräte GmbH) detectors. Solvents for HPLC were obtained as HPLC-grade and degassed by ultrasonication for 15–20 min before use. The gradient elution system used mobile phase A (deionized H_2O + 0.1% trifluoroacetic acid) and mobile phase B (acetonitrile) with a flow of 1 mL/min.

Manual ^{68}Ga Labeling

^{68}Ga was eluted with 5 mL of 0.1 M HCl and subsequently postprocessed online according to a previously published procedure (*15*). For labeling with ethanol-based ^{68}Ga eluate (N5: 90% ethanol/0.9N HCl), 0.10–0.70 μg (0.11–0.74 nmol) of PSMA^{HBED} was added to a mixture of buffer and 0.1–1 mL of ^{68}Ga eluate. The influence of buffer (molarity, volume, pH), amount of ligand, volume of eluate, temperature, and reaction time was investigated.

For clinical application, 0.75–5 μg (0.79–5.28 nmol) of PSMA^{HBED} were added to a mixture of 1,000 μL of 1 M ammonium acetate buffer and 1 mL of ethanol-based ^{68}Ga eluate (1.85-GBq ^{68}Ge/^{68}Ga generator; iThemba Labs). The mixture, with a final pH of 3.9–4.2, was heated for 5 min at 85°C in a closed 10-mL vial followed by sterile filtration and dilution with 10 mL of saline solution.

Synthesis without postprocessing was performed as follows: 1 μg (0.11 nmol) of PSMA^{HBED} was added to a mixture of 600 μL of 3 M ammonium acetate buffer and 2 mL of ^{68}Ga in 0.6N HCl (1.85-GBq ^{68}Ge/^{68}Ga generator; iThemba Labs). The mixture, with a final pH of 4.2, was incubated for 5 min at 40°C in a closed 10-mL vial.

Automated Tracer Synthesis

^{68}Ga obtained from a 1.1-GBq ^{68}Ge/^{68}Ga generator (IGG100; Eckert & Ziegler Strahlen- und Medizintechnik AG) with a TiO_2 matrix was eluted with 0.1N HCl and postprocessed with ethanol/HCl solution according to a method described in the literature (*13,15*). PSMA^{HBED} was labeled by adding aliquots (5, 10, 15 μL = 5, 10, 15 μg = 5.28, 10.56, 15.84 nmol) of a PSMA^{HBED} stock solution (1 mg/mL) to mixtures of postprocessed ^{68}Ga eluate (800 μL) and 1 M NaOAc solution (1.6 mL, pH 7), which corresponds to an ethanol content of 33 vol% of the crude reaction solution using the small radiolabeling synthesizer Modular-Lab eazy (Eckert & Ziegler Strahlen- und Medizintechnik AG) and a temperature of 110°C. Radiochemical yields were determined after a 200- and 300-s reaction time.

Quality Control

TLC was performed with 1-μL aliquots on TLC or ITLC silica gel plates after labeling for 1, 3, 5, and 10 min and subsequently developed in different solvent systems. Analyses were performed using a flat-bed scanner (Instant Imager [Packard Canberra] or Rita Star [Raytest Isotopenmessgeräte GmbH]).

The results were compared with radio-HPLC, which was performed using 2 gradient systems depending on labeling method. The gradient elution system used mobile phase A (deionized H_2O containing 0.1% trifluoroacetic acid) and mobile phase B (100% acetonitrile) and a low rate of 1.0 mL/min. Starting with 100% A/0% B, the gradient was increased to 100% B over 15 min and then returned to the initial gradient conditions within 5 min. The retention time of free ^{68}Ga was 2.8 min; ^{68}Ga-PSMA^{HBED} eluted at 9.5 min.

RESULTS

^{68}Ga Labeling

Currently, fractionated ^{68}Ga eluate is regularly used for radiosynthesis of ^{68}Ga-PSMA^{HBED}. The disadvantage of fractionation is the content of metallic impurities such as ^{68}Ge generator breakthrough and stable ^{68}Zn generated from ^{68}Ga decay (*16*), which are decreased but, in fact, not chemically removed in this case. It is therefore desirable to find optimized conditions using postprocessed ^{68}Ga for ^{68}Ga-PSMA^{HBED} labeling, with the postprocessed ^{68}Ga fraction meeting recommendations for ^{68}Ge/^{68}Ga radionuclide generator eluates as described in the monograph "Gallium (^{68}Ga) Chloride Solution for Radiolabeling" of the European Pharmacopoeia (*17*).

The ^{68}Ga eluate contains measurable activities of the long-lived ^{68}Ge, which is a critical parameter in the context of the routine clinical application of ^{68}Ga-radiopharmaceuticals (*18,19*). Breakthrough in commercial ^{68}Ge/^{68}Ga generators varies over time and by frequency of use. Typical values of initial ^{68}Ge breakthrough (^{68}Ge present in the eluate divided by ^{68}Ga present in the eluate) are on the order of 0.0001%–0.00001%. Over time, this ratio increases because of the decreasing amount of generated and eluted ^{68}Ga. According to the certificates for each individual generator, in particular the GalliaPharm (Eckert & Ziegler Strahlen- und Medizintechnik), there is a guarantee that both initial and permanent ^{68}Ge breakthrough will be less than 0.001%, which is recommended by the European Pharmacopoeia for the synthesis of ^{68}Ga radiopharmaceuticals (*17*). For ^{68}Ge/^{68}Ga generators with higher levels of ^{68}Ge breakthrough, online or offline purification to remove ^{68}Ge from the initial ^{68}Ga eluate is vital. In addition to ^{68}Ge breakthrough, the relatively large volume, high acidity of the eluate, and presence of further metal ion contaminants such as Zn(II) and Fe(III) are problems addressed by these so-called postprocessing procedures. There are no defined limitations to metal contaminants, but research has shown that trivalent metal cations, in particular, can hinder efficient radiolabeling with ^{68}Ga. In addition, a reduction in the labeling yield and in specific activity occur inasmuch as metal contaminants compete with the low amounts of ^{68}Ga (1 GBq ^{68}Ga = 9.731 pmol = 6.61 pg) available for complex formation with the precursor.

Several methods have been developed to reduce metallic impurities and concentrate the eluate, of which variations of cation-exchange resin–based postprocessing have been particularly successful (*13,20*). The initial method pioneered by Zhernosekov et al. using acetone/hydrochloric acid solutions provides high recovery of ^{68}Ga and complete removal of ^{68}Ge, as well as a decrease in acidity, volume, and other metallic impurities (*13*). A suitable and efficient variation is cation-exchange–based postprocessing using ethanol/hydrochloric acid medium (*15*). It equally allows concentration of ^{68}Ga generator eluate, removal of metal impurities, and quantitative removal of ^{68}Ge breakthrough, ensuring that the final injectable radiopharmaceutical fulfills regulatory requirements relating to ^{68}Ge content. A recently published study confirmed the hypothesis that ethanol facilitates incorporation of the radiometal (*21*). The radiolytic protection capability of ethanol additionally benefits a labeling reaction being performed with high activities.

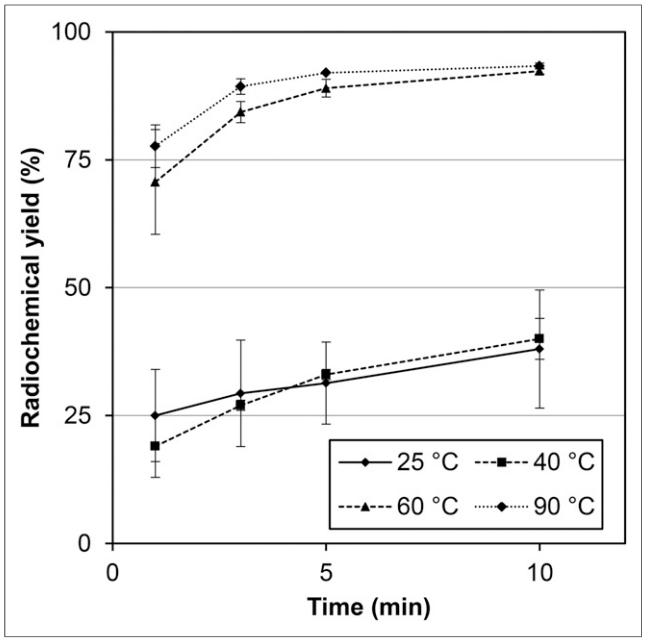

FIGURE 1. Radiolabeling yields with various reaction temperatures using ethanol-postprocessed ^{68}Ga-eluate (0.1 μg/0.11 nmol/0.025 μM PSMA^{HBED}; 1 mL of N5; 3 mL of 0.25 M HEPES, pH 7.5; overall reaction volume, 4 mL; n = 3).

Using a modified labeling method published by Eder et al., optimization was conducted as part of this study (*1*), resulting in the finding that 0.25 M (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) buffer (pH 7.5) is the most suitable system for radiolabeling of PSMA^{HBED} at a very low precursor concentration using 1 mL of postprocessed eluate. Using these conditions, labeling yields were found to be noticeably dependent on reaction temperature. Although HBED is a nonmacrocyclic chelate, ^{68}Ga-complex formation yields are relatively low at ambient temperature, that is, between 25°C and 40°C, and do not exceed 40% after 10 min of reaction time. In contrast, yields and complex formation kinetics are high and fast at elevated temperatures of 60°C and 90°C. At a 10-min reaction time, labeling yields were equivalent for both temperatures (Fig. 1).

Using elevated temperatures and increasing the amount of precursor to 0.7 μg (0.74 nmol), complex formation occurs quickly and reliably. Radiolabeling yields of more than 90% are achievable within 1 min and of more than 95% within 3 min (Fig. 2). These conditions fulfil regulatory requirements without the need for further purification when using a reaction time of 5 min.

In the context of clinical applications, HEPES is not necessarily the buffer of choice, although it is not biologically critical and offers high incorporation of radioactivity and, accordingly, high specific activities. Because there is no monograph of PSMA^{HBED} listed in the pharmacopeia, the radiopharmaceutical has to be purified of HEPES and an additional quality control step is necessary to determine the residual in the final formulation (as described as part of the monograph for ^{68}Ga-DOTATOC). To circumvent additional purification steps, 1 M NH_4OAc solution was used as a buffer medium as part of this study.

Using 900 μL of 1 M NH_4OAc mixed with 1 mL of postprocessed ^{68}Ga-eluate resulted in a labeling pH of 3.9–4.2. A labeling temperature of 85°C was found to be optimal for clinical routine production using ethanol-postprocessed ^{68}Ga eluate. Figure 3 shows radiolabeling kinetics depending on precursor amount using routine production conditions. When using higher activities (>1 GBq) for labeling, more precursor was necessary to obtain satisfactory and reproducible radiochemical yields. Radiolabeling with less than 1 μg (1.1 nmol/0.526 μM) PSMA^{HED} suffers from low reproducibility (±10.3%) and low yields. The use of more than 1 μg (1.1 nmol/0.526 μM) PSMA^{HBED} leads to radiolabeling yields of more than 98% within 5 min of reaction time. In this case, additional purification of the product can be omitted as it already fulfils regulatory requirements. As a variation, radiolabeling of 1 μg (1.1 nmol/0.423 μM) PSMA^{HBED} was performed using fractionated ^{68}Ga eluate at elevated temperature (40°C). In this case, radiolabeling yields of 75.0% ± 5.8% were obtained requiring additional purification of the product before injection. When the results of fractionated and ethanol-based postprocessed ^{68}Ga are compared, the latter are superior.

Transferring the investigated radiolabeling method without further changes to an automated module system (Modular-Lab eazy) was easily achieved. Taking the different heat transmission rate of the reactor into account, higher temperatures than for manual synthesis are necessary. Without further optimization of the conditions toward automatization, radiolabeling yields of 93% ± 3.2% were obtained within 200 s (3.3 min) using the minimum amount of precursor (5 μg/5.28 nmol) recommended by Eckert & Ziegler. An extension of reaction time up to 300 s (5 min) did not affect yields (91% ± 4.5%).

TLC Analytics

So far, quality control of ^{68}Ga-PSMA^{HBED} has been performed by means of radio-HPLC (*1*). Keeping in mind that the time needed for quality control of short-lived nuclides should not exceed the time needed for synthesis, obtaining higher product activities by shortening the time for quality control is crucial when

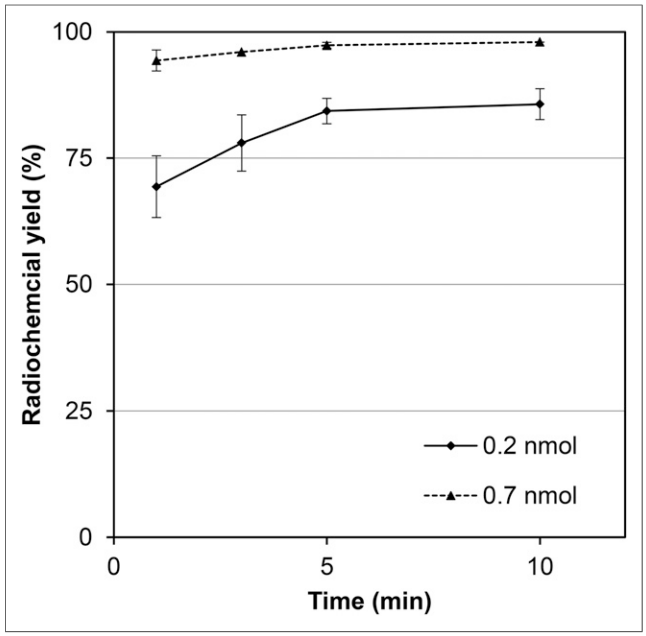

FIGURE 2. Radiolabeling yields with various precursor amounts (90°C; 1 mL of N5, 3 mL of 0.25 M HEPES, pH 7.5; overall reaction volume, 4 mL; n = 3).

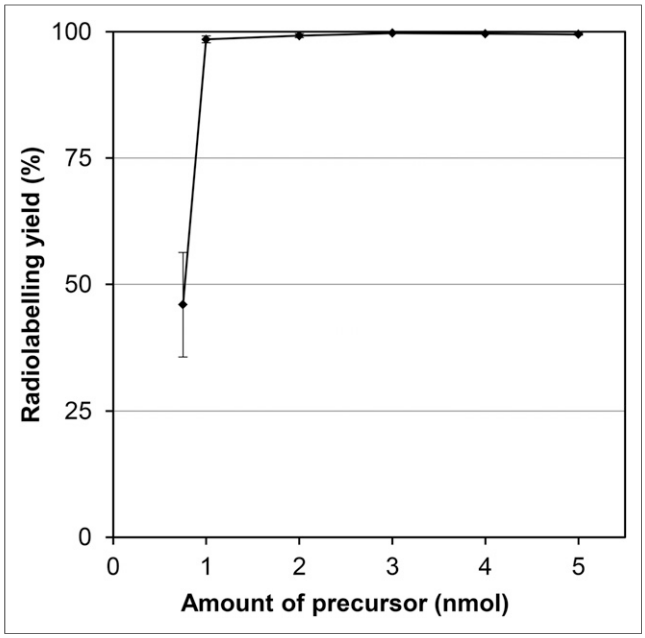

FIGURE 3. Radiolabeling yields with various precursor amounts using postprocessed ^{68}Ga-eluate (85°C; 1 mL of N5; 900 μL of 1 M NH_4OAc, pH 3.9–4.2; $n = 3$).

developing novel procedures for routine clinical application. For example, performing a 20-min HPLC protocol (as suggested by Eder et al. (*1*)) would reduce the absolute ^{68}Ga-PSMA^{HBED} product radioactivity by 18%. In this case, the use of TLC/ITLC appears to be an attractive alternative because the method is generally expected to allow faster but still reliable quality control with little equipment complexity and accordingly cost. Thus, a TLC/ITLC system is required to differentiate between ^{68}Ga and ^{68}Ga-PSMA^{HBED}, making use of the advantages of this quality control method. To find optimum conditions for TLC/ITLC quality control of ^{68}Ga-PSMA^{HBED}, different mobile phases on silica gel 60 and on ITLC silica gel plates were investigated. General separation ability was evaluated with silica gel 60 plates as the stationary phase and several mobile phases. The focus was set on duration of development and separation ability of the investigated TLC systems. The documented R_f values are summarized in Table 1.

With the exception of acetonitrile (*6*) and cyclohexanone (*7*) mixtures, all investigated mobile phases are suitable for separating ^{68}Ga from ^{68}Ga-PSMA^{HBED} on silica gel 60 plates. Comparison with radio-HPLC results confirmed the high reliability of mobile phases 1–3. Altogether, 3 mobile phases were found to be suitable for TLC analytics of ^{68}Ga-PSMA^{HBED} using silica gel 60 plates.

Even though TLC is a reliable low-budget method, the development of the plates takes too long to have an advantage over the established 20-min HPLC procedure. In a second step, ITLC silica gel plates were investigated using mobile phases 1–3 and 5 to shorten the development time of plates in the solvent chamber. All observed R_f values and development times using ITLC silica gel plates as the stationary phase are summarized in Table 1.

Figure 4 shows radio-TLC (left image) and ITLC (right image) images developed in mobile phases 1–3 and 5, with free ^{68}Ga (left lane) directly compared with ^{68}Ga-PSMA^{HBED} (right lane). As anticipated, separation depends on both the mobile phase and the stationary phase because of changes in the interaction dependent on component polarity. As a result, not all investigated mobile phases are suitable for development of both TLC and ITLC, as shown in Table 1 and Figure 4. Altogether, it was possible to find 2 mobile phases (phases 2 and 3) that can be used with silica gel 60 (TLC) and ITLC silica gel plates to determine the radiochemical yield of ^{68}Ga-PSMA^{HBED} for quality control. Quality control was completed in less than 10 min using mobile phases 2 and 3 with ITLC silica gel plates. Compared with more than 15 min for quality control by means of radio-HPLC, this is a fast and easy-to-handle low-budget method with high reliability.

All analytic data obtained by TLC and ITLC silica gel were also verified by means of HPLC (Fig. 5).

DISCUSSION

^{68}Ga-PSMA^{HBED} is a promising new ^{68}Ga-PET tracer that is being increasingly applied for diagnosis of various diseases related to primary prostate cancer and other cancers, such as renal cell carcinoma, that also express PSMA in the neovasculature (*22*). A process of replacing previously used tracers, such as ^{18}F-choline, with ^{68}Ga-PSMA^{HBED} has already begun on the basis of the

TABLE 1
R_f Values for Investigated Mobile Phases Using Silica Gel 60 or ITLC Silica Gel Plates as Stationary Phase

		Silica gel 60			ITLC silica gel		
No.	Mobile phase	R_f (^{68}Ga)	R_f (^{68}Ga-PSMA)	Minutes	R_f (^{68}Ga)	R_f (^{68}Ga-PSMA)	Minutes
1	0.1 M $Na_3C_6H_5O_7$ (pH 4)	0.9–1	0.1	20	0.9–1	0.7–0.8	—
2	MeOH/NH_4OAc (1:1)	0	0.8–0.9	23	0	0.8–0.9	7
3	5% NaCl/MeOH/25% NH_3 (3:1:1)	0	1	18	0	1	5
4	MeOH/0.9% NaCl (9:1)	0	1	—	—	—	—
5	MeOH/0.9% NaCl/1 (mg/mL) EDTA (9:1:0.5)	0–0.1	1	25	0.9–1	0.9	—
6	MeCN/H_2O (1:1)	0–1	0–1	—	—	—	—
7	Cyclohexanone/2 M HCl (20:1)	0	0	—	—	—	—

Development times are given only for applicable solvent systems.

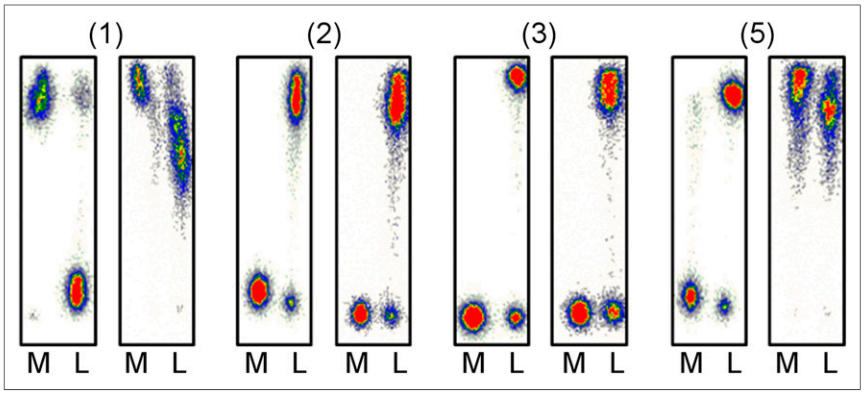

FIGURE 4. Images of radio-TLC (left plate) and ITLC (right plate) developed in mobile phases 1–3 and 5. M = ^{68}Ga; L = ^{68}Ga-PSMA^{HBED}; 1 = 0.1 M $Na_3C_6H_5O_7$, pH 4; 2 = 1:1 MeOH/NH_4OAc; 3 = 3:1:1 5% NaCl/MeOH/25% NH_3; 5 = 9:1:0.5 MeOH/0.9% NaCl/1 (mg/mL) EDTA.

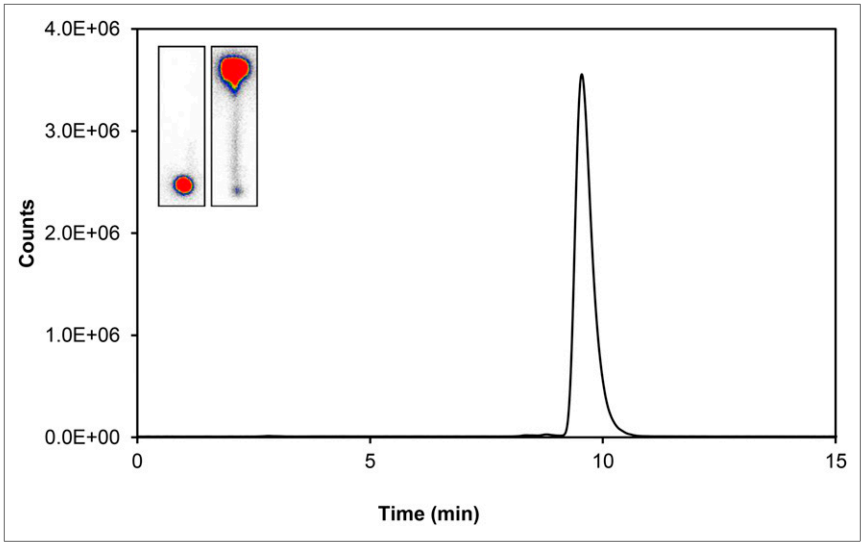

FIGURE 5. Radio-HPLC of ^{68}Ga-PSMA^{HBED} for verification of TLC (left lane, 0.1 M $Na_3C_6H_5O_7$, pH 4) and ITLC (right lane, 1:1 MeOH/NH_4OAc) quality control.

promising results continually being published. Compared with previous ^{18}F-based PET tracers, the synthesis of ^{68}Ga-PSMA^{HBED} exemplifies the advantages of radiometal-based PET tracers. One may soon expect the availability of kit-analog preparations, as recently reported for a ^{68}Ga-octreotide derivative (*23*).

However, those syntheses should be robust and reliably guarantee radiochemical labeling yields higher than 99%, making subsequent purification steps unnecessary. In the case of ^{68}Ga-radiopharmaceuticals, an additional isolation of ^{68}Ge via postprocessing procedures or quality control for ^{68}Ge breakthrough in the product synthesized should become redundant.

The present study was able to modify the synthesis of ^{68}Ga-PSMA^{HBED} by adopting established ^{68}Ge/^{68}Ga generator postprocessing methods to eliminate ^{68}Ge breakthrough before ^{68}Ga-labeling. Because acetone- and ethanol-driven cation-exchange postprocessing pathways are online, fast, and almost quantitative, the yield of ^{68}Ga-PSMA^{HBED} labeling is not affected. Labeling yields of more than 99% are achieved at optimized conditions, and product becomes available within 5 min after generator elution—including postprocessing. The synthesis is transferable to automated modules such as the Modular-Lab eazy, achieving acceptable yields even at a lower pH. It was possible to develop fast and reliable TLC- and ITLC-based methods that provide results comparable to the established HPLC method. This is important in clinical applications for which rapid and stable quality control is indispensable. Because the gain in product activity due to the short synthesis period will decrease whenever longer periods are required for quality control (such as a 20-min HPLC protocol as suggested by Eder et al. (*1*)), the new ITLC quality control is of special importance and can be terminated within 5 min using a fast, reliable, low-cost radio-ITLC method with little equipment complexity. Analytic data obtained with this ITLC system are confirmed by HPLC.

CONCLUSION

Adaptation of the initially described synthesis of the ^{68}Ga tracer (for nonpostprocessed ^{68}Ge/^{68}Ga generator eluates) to state-of-the-art procedures for cation-exchange–based eluate purifications has been straightforward. Radiolabeling yields are nearly quantitative. The synthesis is completed within 5 min, providing labeling yields of more than 95% and specific activities of more than 326 ± 20 MBq/nmol, making subsequent product purification obsolete. The synthesis, as well as the TLC/ITLC quality control methods, was successfully implemented in the systematic clinical protocols for over 200 patient studies.

DISCLOSURE

Eckert & Ziegler Eurotope GmbH (Berlin, Germany) provided the automated system Modular-Lab eazy. No other potential conflict of interest relevant to this article was reported.

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