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Optimization of labeling PSMA^{HBED} with ethanol-post-processed ^{68}Ga and its quality control systems

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ABSTRACT

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 lesions of prostate cancer due to the high expression of PSMA in prostate carcinoma cells as well as bone metastases and lymph nodes related to the disease. The aim of this work was the optimization of the labeling of ^{68}Ga-PSMA^{HBED} using the efficient cation exchange (CEX) post-processing of ^{68}Ga as well as the development of a TLC-based quality control system.

Labeling was optimized for online ethanol post-processed ^{68}Ga eluate investigating various parameters, such as buffer molarity (0.1-1 M), temperature (25-90°C), tracer amount (0.11-0.74 nmol) and labeling time. In addition purification of the crude product using a STRATA-X cartridge was tested. For radio-TLC quality control various mobile phases were analyzed using silica gel 60 plates and results were validated using HPLC. The most superior mobile phases were also applied on ITLC-SG-plates.

Using optimized conditions labeling yields of > 95% were obtained within 10 min when applying the ethanol-based post-processing using PSMA^{HBED} amounts as low as 0.1 nmol. Higher precursor concentration (0.7 nmol) further increased labeling and quantitative yields to >98% within 5 min. In clinical routine patient batches (> 200 applications) with radiochemical purity > 98 % and specific activities of 326 ± 20 MBq/nmol are obtained reproducibly.

Performing TLC quality control on silica gel 60 plates, four mobile phases with suitable separation properties and complementary R_f values were identified. Two systems show equivalent separation on ITLC-SG-plates, with ITLC analysis finished within 5 min in contrast to the TLC system (20 min).

Labeling of PSMA^{HBED} was optimized for CEX post-processing methods ensuring almost quantitative labeling and high nuclide purity of final ^{68}Ga-PSMA^{HBED}, making subsequent purification steps unnecessary. The new radioTLC-method allows quality control in a short time using a fast, reliable, low

cost method with little equipment effort. Using this approach, the synthesis is easily adopted by automated synthesis modules such as e.g. the EZAG Modular-Lab eazy.

KEYWORDS: ^{68}Ga, PSMA, generator post-processing, quality control, ITLC

INTRODUCTION

Prostate-specific membrane antigen (PSMA) is a cell surface protein with increased expression on nearly all prostate cancer cells compared to other PSMA expressing tissues such as kidney, proximal small intestine or salivary glands[1–3]. As PSMA expression is restricted to the prostate and the cell surface at all stages of disease it therefore holds promise as target for specific imaging and therapy of prostate cancer and neovasculature[4–6]. It has recently been demonstrated that low-molecular peptido-mimetic radiopharmaceuticals are clinically very attractive as it is possible to image prostate cancer lesions with high contrast and higher sensitivity compared to ^{18}F-Choline-PET/CT[7–10]. Non-invasive imaging of increased PSMA expression provides important information related to the stage of prostate cancer and 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 prostate cancer imaging agent[1, 8]. It is an 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 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 in order 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 HPLC for quality control. This report describes radiolabeling of PSMA^{HBED} using cation exchange-based (CEX) post-

processing methods for manual synthesis as well as the use of an automated module followed by the development of a TLC- and ITLC-based quality control system.

EXPERIMENTAL PROCEDURES

Only the highest reagent grade chemicals and Trace-Select 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 years old) from Cyclotron Co. Ltd. (Obninsk, Russian Federation) and from an initially 1.85 GBq ^{68}Ge/^{68}Ga generator (new) from iThemba Labs (Cape Town, South Africa).

BioRad AG 50W-X4 (200 – 400 mesh) cation-exchange (CEX) resin was used to prepare a micro-chromatography column (50 mg resin, 2 mm inner diameter, 5 mm length). Also Varian Bond Elut-SCX was used. Labeling reactions were carried out in 11 mL glass vials (Mallinckrodt) using a blockthermostat (TK13, Ditabis) for temperature control and agitation. Purification was performed with 30 mg C-18 cartridges (Phenomenex Strata-X Tubes). Activity measurement was performed using a Curie-Meter (ISOMED 2010, Nuklear-Medizintechnik Dresden GmbH). pH measurement was performed using a calibrated pH-meter (SevenEasy pH, Mettler-Toledo). TLC plates (aluminum-backed silica gel 60, Merck) and ITLC-SG plates (Varian) were analyzed using a flat-bed scanner (Instant Imager, Canberra Packard; miniGita, Raytest-Isotopenmessgeräte GmbH, Straubenhardt, Germany).

RP-HPLC using a LiChrosphere 100-RP18EC column (5 mm, 250 x 4 mm) was used to quantify the radiochemical purity of ^{68}Ga-PSMA. HPLC was equipped with a Hitachi L-7100 pump system coupled with UV (Hitachi L-7400) and radiometric (Gamma Raytest-Isotopenmessgeräte GmbH, Straubenhardt, Germany) detectors. Solvents for HPLC were obtained as HPLC grade and degassed by ultrasonication for 15–20 min before use. The gradient elution system utilized mobile phase A (deionized H_2O + 0.1 % TFA) 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 post-processed online according to a previously published procedure[15]. For labeling with ethanol-based ^{68}Ga eluate (N5: 90 % ethanol/0.9 N HCl) 0.10-0.70 µg (0.11-0.74 nmol) of PSMA^{HBED} were added to a mixture of buffer and 0.1-1 mL of ^{68}Ga eluate. The influences of buffer (molarity, volume, pH), amount of ligand, volume of eluate, temperature and reaction time were investigated.

For clinical application 0.75-5 µg (0.79-5.28 nmol) PSMA^{HBED} were added to a mixture of 1000 µL 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 final pH 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 saline solution.

Synthesis without post-processing 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 0.6 N HCl (1.85 GBq ^{68}Ge/^{68}Ga generator, iThemba Labs). The mixture with final pH 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, Berlin, Germany) with a TiO_2 matrix, was eluted with 0.1 N HCl and post-processed with ethanol/HCl solution according to literature[13, 15]. Labeling of PSMA^{HBED} was performed 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 post-processed ^{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 LabEazy (Eckert & Ziegler Strahlen- und Medizintechnik AG, Berlin, Germany) and a temperature of 110°C. Radiochemical yields were determined after 200 and 300 s reaction time.

Quality control

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

Results were compared to radioHPLC which was performed using two gradient systems depending on labeling method. The gradient elution system utilized mobile phase A (deionized H_2O containing 0.1 % TFA) and mobile phase B (100 % acetonitrile) and flow 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 R_t = 2.8 min, ^{68}Ga-PSMA^{HBED} eluted at 9.5 min.

RESULTS & DISCUSSION

^{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 post-processed ^{68}Ga for ^{68}Ga-PSMA^{HBED} labeling with the post-processed ^{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 pharmacopeia[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]. The breakthrough of commercial ^{68}Ge/^{68}Ga generators varies among suppliers, over time and frequency the generators are used. Typical values of initial ^{68}Ge breakthrough (^{68}Ge present in the eluate divided by ^{68}Ga present in the

eluate) are in the order of 0.0001-0.00001 %. Over a period of time this ratio increases due to the decreasing amount of generated and eluted ^{68}Ga. According to certificates of each individual generator, in particular the GalliaPharm (Eckert & Ziegler Strahlen- und Medizintechnik, Berlin, Germany) guarantees both initial and permanent ^{68}Ge breakthrough 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, an online or offline purification to remove ^{68}Ge from initial ^{68}Ga eluate is vital. In addition to ^{68}Ge breakthrough the relatively large volume, high acidity of the eluate and the presence of further metal ion contaminants e.g. Zn(II) or Fe(III) are problems addressed with these so-called post processing procedures. There are no defined limitations to metal contaminants but research has shown particularly trivalent metal cations can hinder efficient radiolabeling with ^{68}Ga. In addition reduced labeling yields and specific activities 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 (CEX) resin based post-processing 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 decreasing acidity, volume and other metallic impurities[13]. A suitable and efficient variation, is the cation-exchange based post-processing using ethanol/hydrochloric acid media[15]. It equally allows concentration of ^{68}Ga generator eluate, removal of metal impurities and quantitative removal of ^{68}Ge-breakthrough, ensuring the final injectable radiopharmaceutical fulfilling regulatory requirements relating to ^{68}Ge content. A recently published study confirmed the hypothesis of ethanol facilitating incorporation of the radio metal[21]. The radiolytic protection capability of ethanol additionally adds benefit to a labeling reaction being performed with high activities.

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

Figure 1

Using elevated temperatures and increasing the amount of precursor to 0.7 µg (0.74 nmol), complex formation occurs fast and reliable. Radiolabeling yields of >90 % are achievable within 1 min and >95 % within 3 min (Figure 2). Using these conditions regulatory requirements are fulfilled without the necessity of further purification when using a reaction time of 5 min.

Figure 2

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

Using 900 µL of 1 M NH_4OAc mixed with 1 mL of post-processed ^{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 post-processed ^{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 > 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 prior to injection. When comparing results of fractionated and ethanol-based post-processed ^{68}Ga the latter shows superior results.

Figure 3

Transferring the investigated radiolabeling method without further changes to an automated module system (Modular LabEazy) was easily achieved. Taking the different heat transmission rate of the reactor into account higher temperatures are necessary compared to manual synthesis. Without further optimization of the conditions towards 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 show an effect on yields (91 ± 4.5 %).

TLC-Analytics

So far, quality control of ^{68}Ga-$PSMA^{HBED}$ has been performed by means of RadioHPLC[1]. Keeping in mind that the time needed for QC of short-lived nuclides should not exceed the time needed for synthesis.

Obtaining higher product activities by shortening time necessary for QC is a crucial parameter when developing novel routine procedures for clinical application. For example, performing a 20 minute HPLC protocol (as suggested by Eder et al.) would reduce the absolute ^{68}Ga-PSMA^{HBED} product radioactivity by 18 percent. In this case the use of TLC/ITLC appears as an attractive alternative as the method is generally expected to allow a faster but still reliably quality control with little equipment effort and accordingly low 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. In order to find optimum conditions for TLC/iTLC QC of ^{68}Ga-PSMA^{HBED}, different mobile phases on Silica Gel 60 and on ITLC-SG plates were investigated. General separation ability was evaluated with Silica Gel 60 plates as stationary phase and several mobile phases. 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.

Table 1

With the exception of acetonitrile (6) and cyclohexanone (7) mixtures, all investigated mobile phases are suitable to separate ^{68}Ga from ^{68}Ga-PSMA^{HBED} on Silica Gel 60 plates. Comparison with radioHPLC results confirmed high reliability of mobile phase 1-3. Altogether three 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 in order to have an advantage over the established 20 min HPLC procedure. In a second step ITLC-SG plates were investigated using mobile phases 1-3 and 5 to shorten development time of plates in the solvent chamber. All observed R_f values and development times using ITLC-SG plates as stationary phase are summarized in Table 1.

Figure 4 shows radioTLC (left image) and ITLC (right image) images developed in mobile phases 1-3 and 5 with free ^{68}Ga (left lane) directly compared to ^{68}Ga-PSMA^{HBED} (right lane). As anticipated, separation

depends on both the mobile phase and the stationary phase due to 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 1and Figure 4. Altogether it was possible to find two mobile phases (No. 2, 3) which can be used with Silica Gel 60 (TLC) and ITLC-SG plates to determine the radiochemical yield of ^{68}Ga-PSMA^{HBED} for quality control. Completion of quality control was achieved in less than 10 min utilizing mobile phase`s No. 2 and 3 with ITLC-SG plates. Compared to more than 15 min for quality control by means of radioHPLC, this is a fast and easy to handle low-budget method with high reliability.

Figure 4

All analytical data obtained by TLC and ITLC-SG were also verified by means of HPLC (Figure 5).

Figure 5

CONCLUSION

^{68}Ga-PSMA^{HBED} is a promising new ^{68}Ga-PET tracer which is increasingly applied for diagnosis of various diseases related to primary prostate cancer and other cancers, such as renal cell carcinoma which 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 started based on promising results being published continuously. Compared to previous ^{18}F-based PET tracers, the synthesis of ^{68}Ga-PSMA^{HBED} exemplarily demonstrates the advantages of radiometal-based PET tracers. One may soon expect the availability of kit-analogue preparations, as recently reported for a ^{68}Ga-octreotide derivative[23].

However, those syntheses should be robust and reliably guarantee radiochemical labeling yields > 99%, making subsequent purification steps unnecessary. In the case of ^{68}Ga-radiopharmaceuticals, an

additional isolation of ^{68}Ge via post-processing procedures or quality control for ^{68}Ge breakthrough in the product synthesized should be made redundant.

The present study was able to modify the synthesis of ^{68}Ga-PSMA^{HBED} by adopting established ^{68}Ge/^{68}Ga generator post-processing methods, used for, eliminating ^{68}Ge breakthrough prior to ^{68}Ga-labeling. As acetone- and ethanol-driven cationic CEX post-processing pathways are online, fast and almost quantitative, ^{68}Ga-PSMA^{HBED} labeling is not affected in terms of yield. Labeling yields of >99% are achieved at optimized conditions and product availability is granted within 5 minutes after generator elution – including post-processing. The synthesis is transferable to automated synthesis modules such as e.g. the EZAG Modular-Lab eazy, achieving acceptable yields even at lower pH. It was possible to develop fast and reliable TLC- and ITLC-based methods, which provide results comparable to the established HPLC method. This is very important in the context of clinical applications where rapid and stable quality control is indispensable. As the gain in product activity due to the short synthesis period would decrease whenever longer periods would be required for quality control (such as e.g. a 20 minutes HPLC protocol as suggested by Eder et al.[1]), the new ITLC quality control is of special importance and can be terminated within 5 minutes, using a fast, reliable, low cost radio ITLC-method with little equipment effort. Analytical data obtained with this ITLC system are confirmed by HPLC.

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

AUTHOR INFORMATION

Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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FIGURES

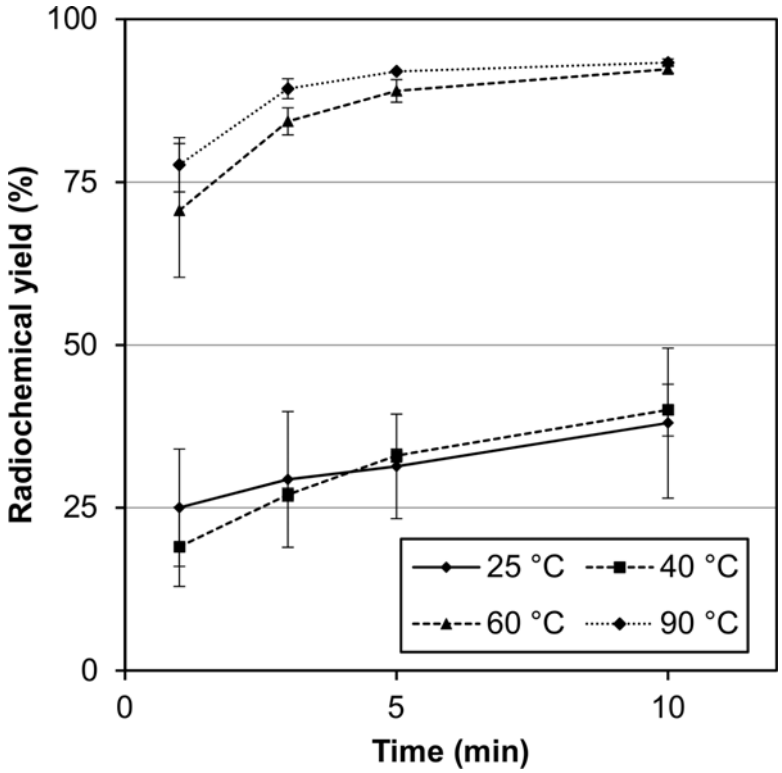

Figure 1: Radiolabeling yields with various reaction temperatures using ethanol post-processed ^{68}Ga-eluate (0.1 µg/0.11 nmol/0.025 µM PSMA^{HBED}, 1 mL N5, 3 mL of 0.25 M HEPES pH 7.5, overall reaction volume = 4 mL; n = 3).

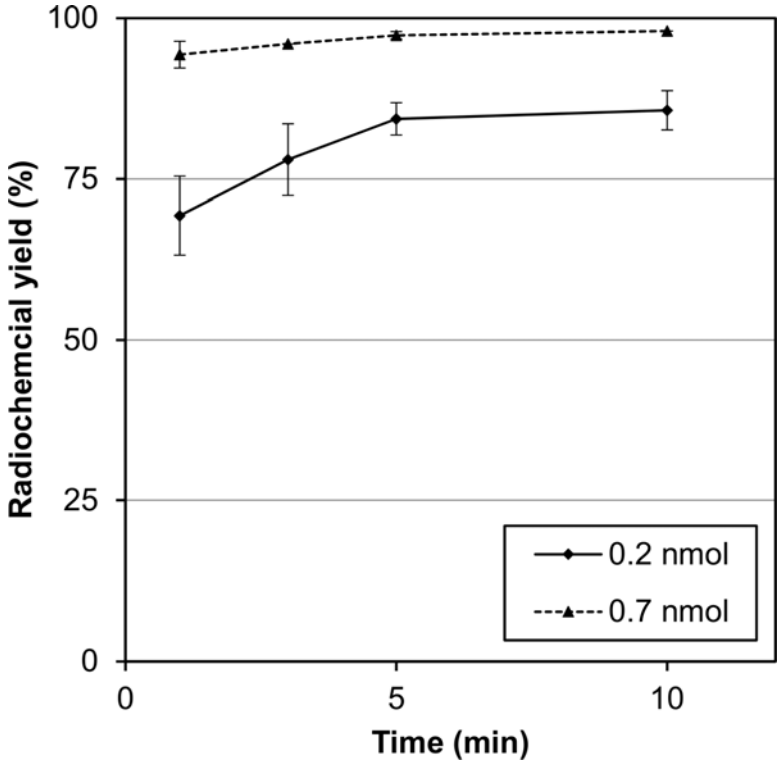

Figure 2: Radiolabeling yields with various precursor amounts (T = 90°C, 1 mL N5, 3 mL of 0.25 M HEPES pH 7.5, overall reaction volume = 4 mL; n = 3).

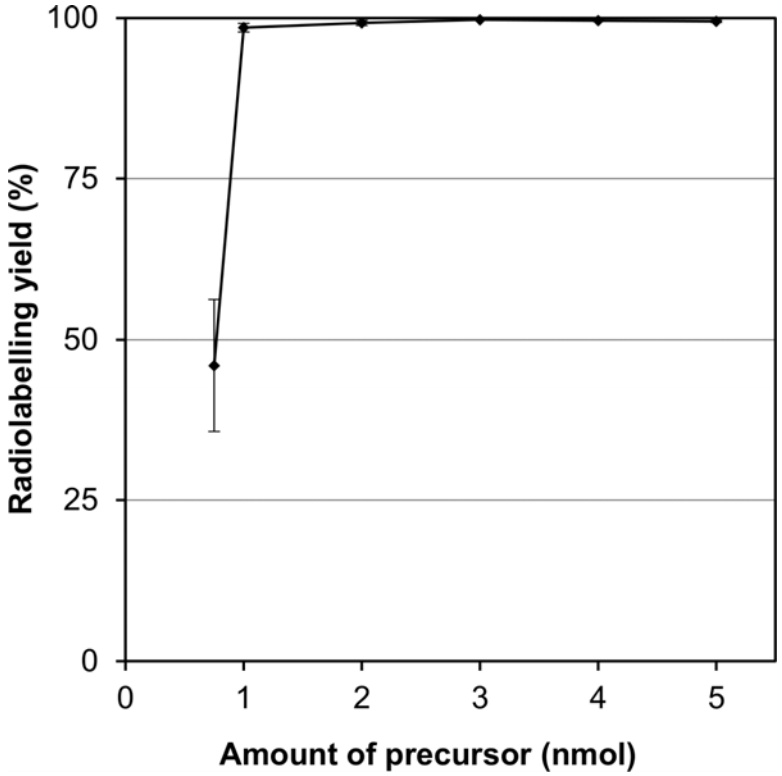

Figure 3: Radiolabeling yields with various precursor amounts using post-processed ^{68}Ga-eluate (85°C, 1 mL N5, 900 µL of 1 M NH_4OAc, pH 3.9-4.2; n = 3).

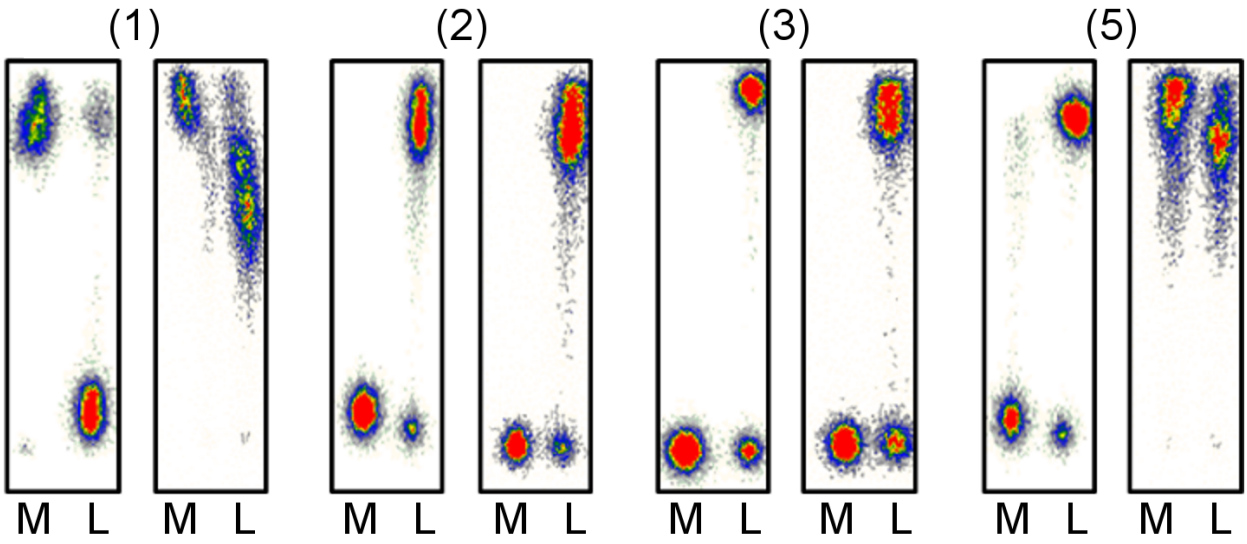

Figure 4: Images of radioTLC (left plate) and ITLC (right plate) developed in the mobile phases 1-3 and 5. M = ^{68}Ga; L = ^{68}Ga-PSMA^{HBED}; 1 = 0.1 M $Na_3C_6H_5O_7$ pH 4; 2 = MeOH/ NH_4OAc 1:1; 3 = 5 % NaCl/ MeOH/25 % NH_3 3:1:1; 5 = MeOH/ 0.9 % NaCl /1 $\frac{mg}{ml}$ EDTA (9:1:0.5).

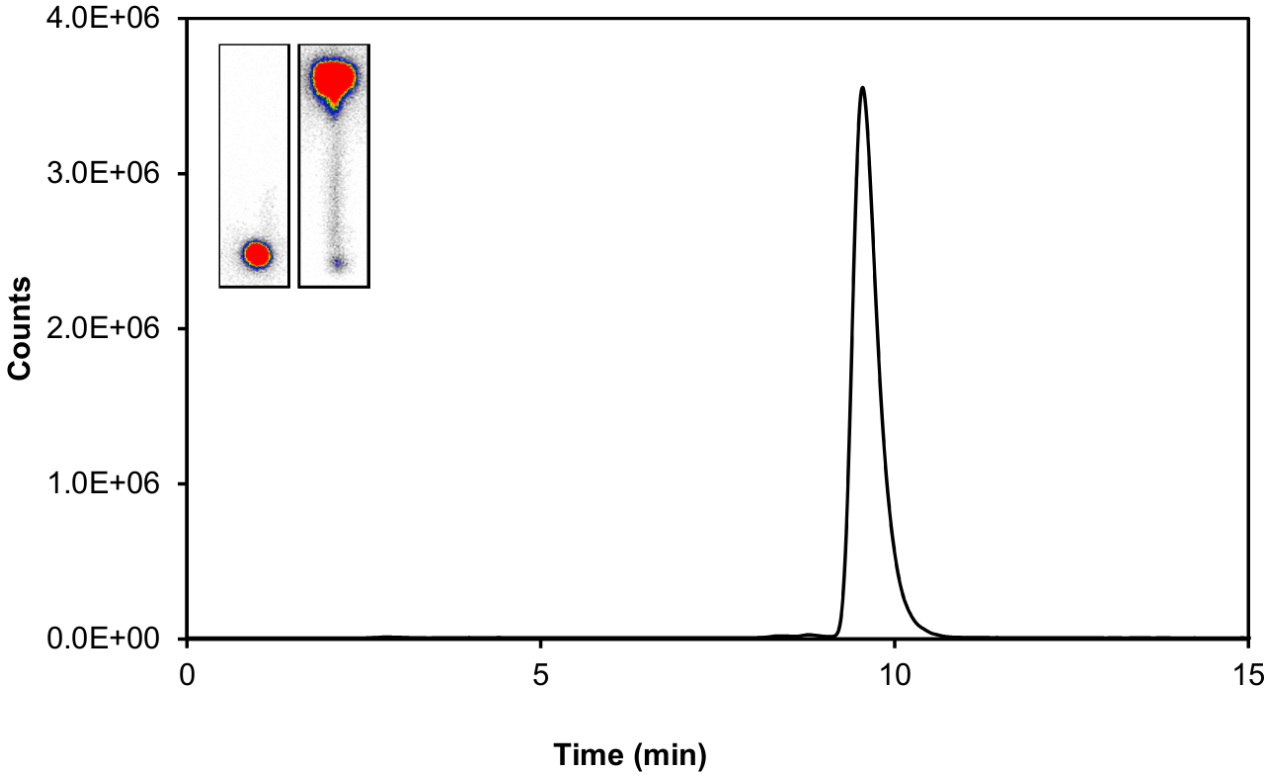

Figure 5: RadioHPLC of ^{68}Ga-PSMA^{HBED} for verification of TLC (left lane, 0.1 M $Na_3C_6H_5O_7$ pH 4) and ITLC (right lane, MeOH/ NH_4OAc 1:1) quality control.

TABLES

Table 1: R_f values for investigated mobile phases using Silica Gel 60 or ITLC-SG plates as stationary phase. Development times were only given for applicable solvent systems.

No.	*Mobile phase*	*Silica Gel 60*			*ITLC-SG*		
		R_f(^{68}Ga)	R_f(^{68}Ga-PSMA)	Time [min]	R_f(^{68}Ga)	R_f(^{68}Ga-PSMA)	Time [min]
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 M 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	-	-	-	-