ABX advanced biochemical compounds

Fully automated synthesis of ^{68}Ga-labelled peptides using the IBA Synthera® and Synthera® Extension modules

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Introduction

The application of ^{68}Ga-labelled peptides for tumour diagnosis is a fast-growing sector in nuclear medicine.[1] ^{68}Ga-DOTA-TATE, ^{68}Ga-DOTA-NOC and ^{68}Ga-DOTA-TOC are administered on a routine basis in PET centers world-wide for the detection of neuroendocrine tumors. A second boost came just recently with the invention of ^{68}Ga-PSMA-11 as a tracer for the imaging of prostate cancer.[2] We have recently described the automated production of ^{68}Ga-CPCR4.2 and other ^{68}Ga-tracers using a NaCl-based cationic purification of the generator eluate.[3]

Aim

Our aim was to develop a disposable kit system which allows the automated production of a variety of ^{68}Ga-labelled peptides using the IBA Synthera® and Synthera ® Extension.

Methods and Materials

^{68}Ge/^{68}Ga-generator In initial trials, an IGG100 from Eckert & Ziegler Eurotope GmbH was used. The mean activity per elution was 200 to 300 MBq. In the first experiments, elution of the generator and transfer to the IFP™ was performed manually. In the late stage of development, an Eckert & Ziegler Radiopharma GmbH GalliaPharm generator (1850 MBq) was used which was eluted with the Extension module. Both generators were eluted with metal-free 0.1 M HCl.

Chemistry $^{68}Ga^{3+}$ was collected on a PS-H$^+$ (M) cartridge and eluted with 5 M NaCl in 0.1 HCl into the reaction vessel which was already pre-loaded with 1.5 M HEPES or acetate buffer solution and the respective peptide. After heating at 95 °C for 7 minutes, the solution was transferred to a pre-conditioned Light C18 cartridge. In case HEPES buffer was used, the cartridge was thoroughly rinsed with water. When acetate buffer was used during labelling, the C18 cartridge was rinsed only once. The final product was then eluted with ethanol-water 1:1 solution. The final product was sterile-filtered and further diluted with phosphate-buffered saline (PBS).

IFP™ For the ^{68}Ga-process, the standard nucleophilic IFP was re-build in order to avoid contamination of the Synthera® module. The activity inlet at the back of the module was not used. The incoming activity was loaded onto the IFP via an extra tubing. HEPES was manually loaded into the reaction vessel using an extra tubing, too (see Figures 1 and 2).

Peptides The following ABX-peptides were used: PSMA-11 (9921), PSMA-617 (9933), DOTA-TATE (9772), DOTA-NOC (9712). For the DOTA-peptides, bulk vials containing 1 mg were used. The vials were filled with 1000 µl metal-free water and the required amounts were withdrawn using an Eppendorf pipette. For PSMA-11, pre-filled vials containing 10 µg were pooled and the precursor was diluted with HEPES or acetate buffer.

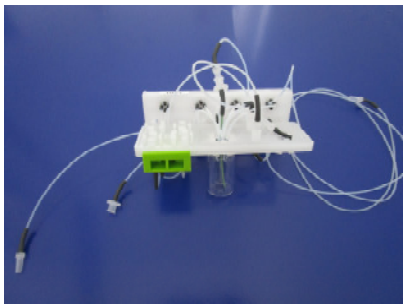

Figure 1 IFP for ^{68}Ga-labelling of peptides using the IBA Synthera® module.

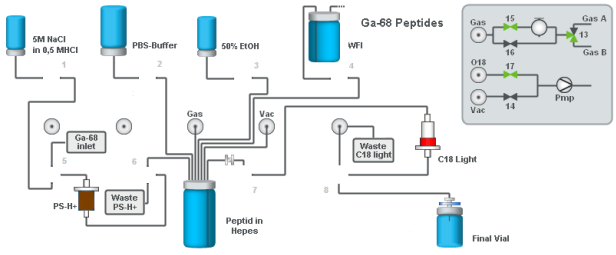

Figure 2 Background picture of the ABX ^{68}Ga-process on the IBA Synthera® module.

Quality control

The radiochemical purity of the labelled peptides was confirmed by HPLC and TLC analysis. For the detection of free $^{68}Ga^{3+}$, HPLC was performed using a Chromolith RP-18 column (3 µm, 4.6 x 100 mm) on a Dionex U3000 UHPLC system equipped with a γ-detector from Berthold Technologies. The solvents A) water + 0.1% TFA and B) acetonitrile were used. The following gradient was used for HPLC analysis: 0-1 min 90% A, 1-6 min 90% A to 100% B, 6-8 min 100% B, 8-9 min to 90% A. Flow rate: 3.0 mL / min.

For the detection of colloidal ^{68}Ga-species, TLC with MeOH/1M NH_4OAc 1:1 was performed on iTLC-SG stripes.

HEPES was detected using the spot test method from Ph. Eur. 2482. Additionally, the absence of HEPES was confirmed by HPLC using a SIELC Obelisc N column and a 85:15-mixture of 0.05% H_3PO_4 and acetonitrile as eluent.

Results

All peptides were obtained in good yields and high radiochemical purity. The radiochemical purity was >98% in all cases with the characteristic double-peak shape for ^{68}Ga-PSMA-11 (see Figure 3). In all cases HEPES was used, the amount of HEPES was <200 µg/V with V = 14 ml.

Entry	Peptide	Yield	Radiochemical purity
1	50 µg DOTA-TATE	>55%	>99%
2	50 µg DOTA-NOC	>55%	>99%
3	20 µg PSMA-11	>55%	>98%
4	50 µg PSMA-617	>55%	>99%

Table 1

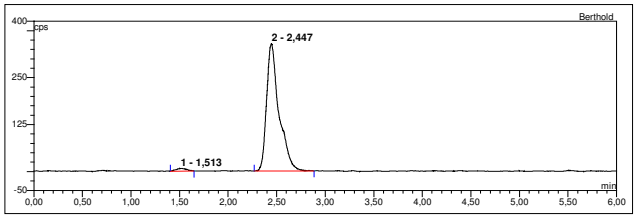

Figure 3 Radio-HPLC chromatogram from the production of ^{68}Ga-PSMA-11

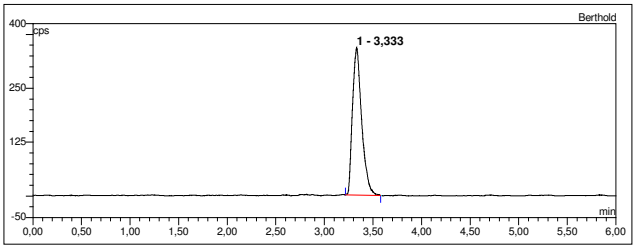

Figure 4 Radio-HPLC chromatogram from the production of ^{68}Ga-DOTA-NOC

Conclusion

We have developed a disposable kit system which allows the automated production of ^{68}Ga-peptides on the IBA Synthera® either using HEPES or acetate buffer. The synthesis includes a pre-purification of the generator eluate by trapping the activity on a cation exchange cartridge. Using 50 µg of DOTA-peptide or 20 µg of PSMA-11, the ^{68}Ga-labelled compounds were obtained in high yield and purity.

The present disposable kit is suitable for the GMP-compliant production of ^{68}Ga-peptides using commercial generators eluted with 0.1 M HCl.

IFPs and reagents for ^{68}Ga-synthesis will be available from ABX soon. Please contact sales@abx.de.

References [1] S. R. Banerjee, M. G. Pomper, *Appl. Radiat. Isotop.* **2013**, *76*, 2–13. [2] J. J. Morigi, P. D. Stricker, P. J. van Leeuwen, R. Tang., et al., *J. Nucl. Med.* **2015**, *56*, 1185–1190. [3] R. Martin, S. Juettler, M. Mueller, H.-J. Wester, *Nucl. Med. Biol.* **2014**, *41*, 84–89. [4]