ABX advanced biochemical compounds

Automated production of ^{68}Ga-labelled peptides using the GE TRACERlab® MX, Ora Neptis® and Siemens Explora® One synthesizers

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Introduction

The application of ^{68}Ga-labeled peptides for tumour diagnosis is a fast-growing sector in nuclear medicine.[1] ^{68}Ga-DOTATATE, ^{68}Ga-DOTANOC (see Figure 1) and ^{68}Ga-DOTATOC are administered on a routine basis in PET centers world-wide. As a consequence, robust labelling technologies and a variety of dedicated synthesis modules have been developed for routine production of these important tracers.[2] We have recently described the automated production of ^{68}Ga-CPCR4.2 and other ^{68}Ga-tracers on the SCINTOMICS GRP module using a NaCl-based cationic purification of the generator eluate.[3] Further interesting cassette-based synthesis modules are the GE TRACERlab® MX, the Ora® Neptis and Siemens Explora® One. In the past, we have established a series of kits which use SPE purification.[4] However, to the best of our knowledge, there is no report describing the ^{68}Ga-labelling of peptides using the MX module. Interestingly, the Ora Neptis® and the Siemens Explora® One can use the same disposable cassettes as the MX module. Therefore, a kit system developed on the MX would potentially allow easy adaption to the Neptis® and Explora® One modules.

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 GE TRACERlab® MX, Ora Neptis® and Siemens Explora® One synthesizers.

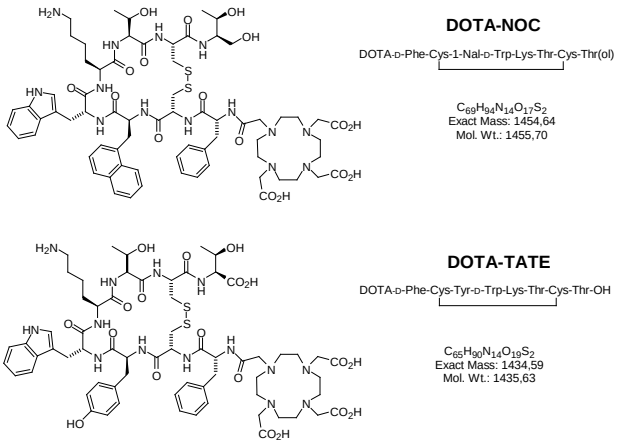

Figure 1: DOTANOC and DOTATATE.

Methods and Materials

^{68}Ge/^{68}Ga-generator An iThemba Labs (South Africa) generator based on a SnO_2 column with an initial activity of 370 MBq (10 mCi) was used. During the present study, the generator was eluted with metal-free 1.0 M HCl and provided 150 – 180 MBq on average. Furthermore, a metal-free generator from ITG GmbH (Garching, Germany) with an initial activity of 1.8 GBq (50 mCi) was used. On average, 900 MBq ^{68}Ga^{3+} were obtained after elution with metal-free 0.05 M HCl. To further support the automated elution of this generator (high back-pressure of the generator), 1.25 bar of nitrogen pressure was applied to the 0.05 M HCl storage vessel.

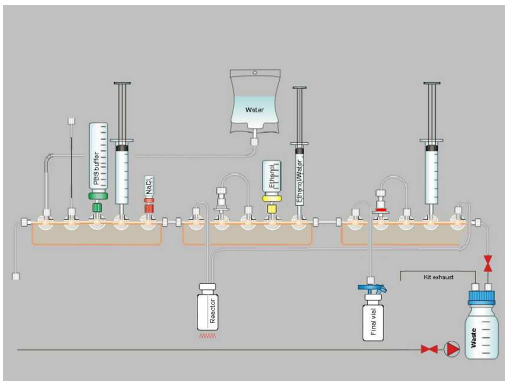

Figure 1: Hardware and reagent kit for automated ^{68}Ga-labelling of peptides using the GE TRACERlab® MX module.

Experimental details

The generator was eluted using the syringe driver from the module. After 10 ml of eluate were collected, the activity was withdrawn from the generator eluate by trapping on a PS-H+ cartridge and the solution was passed into waste. Elution of ^{68}Ga^{3+} from the cartridge was performed with 5 M NaCl. The labelling time and amount of peptide have been varied (20 to 30 µg, 5 to 8 min). The temperature was kept at 125 °C. Purification was performed using a pre-conditioned Light C18 cartridge. The product was eluted with 2 ml of a 1:1-mixture of ethanol/water into the final vial by passing through a sterile filter. The product solution was further diluted with phosphate buffered saline which was also dispensed through the sterile filter. The results of these optimization runs can be found in Tables 1 and 2.

Entry	Labelling time [min]	Amount of peptide	n.d.c yield
1	5	20 µg DOTATATE	54.3 ± 3%
2	6	20 µg DOTATATE	64.0 ± 7%
3	8	20 µg DOTATATE	54.8 ± 2%
4	6	30 µg DOTATATE	69.0 ± 2%
5	6	30 µg DOTANOC	72.6 ± 4%

Table 1: Overview of non decay corrected yields using the iThemba Labs generator (eluted with 1.0 M HCl); labelling was performed at 125 °C in a p lastic reactor.

It was found that 30 µg DOTANOC or DOTATATE gave the best results. Moreover, by increasing the labelling time from 5 to 6 min, a significant increase of yield was observed.

With these optimised conditions in hand, we performed the same sequence using the ITG generator which is eluted with 0.05 M HCl (Table 2). The elution of ^{68}Ga^{3+} from the generator was supported by an overpressure onto the HCl storage vessel (1.25 bar of helium gas). The yields obtained for production of ^{68}Ga-DOTATATE and ^{68}Ga-DOTANOC are comparable to those obtained with the IDB generator (Table 1, entries 5 and 6).

Entry	Peptide	n.d.c yield
1	DOTATATE	71.0 ± 3%
2	DOTANOC	76.2 ± 4%

Table 2: Overview of non decay corrected yields using the ITG generator (eluted with 0.05 M HCl).

Quality control

The radiochemical purity of the labelled peptides was confirmed by HPLC analysis (>99.8%; see Figure 3). HPLC was performed using a ACE 3 C18 column (3 µm, 4.6 x 50 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 +0.1% TFA were used. The following gradient was used upon HPLC analysis: 0-5 min 100% A, 5-20 min 100% A to 100% B, 20-30 min 100% B, 30-32 min 100% B to 100% A, 32-42 min 100% A. Flow rate: 0.6 mL / min.

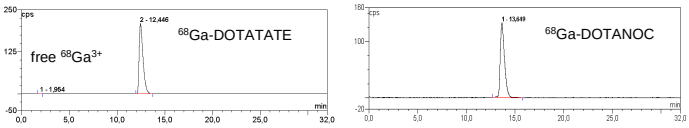

Figure 3: Radio-HPLC chromatograms from the production of ^{68}Ga-DOTATATE and ^{68}Ga-DOTANOC after SPE purification using the GE TRACERlab MX module.

Results

We have developed a disposable kit system which allows the automated production of ^{68}Ga-DOTATATE and ^{68}Ga-DOTANOC on the GE TRACERlab® MX. Since the Ora Neptis® and Siemens Explora® One synthesizers use the same cassettes and sequences, an adaption of the present method to these modules is straightforward. The synthesis includes a pre-purification of the generator eluate by trapping the activity on a cation exchange cartridge. Using 30 µg of DOTATATE or DOTANOC, the ^{68}Ga-labelled peptides were obtained in high yield, independent from the generator used.

Conclusion

The present disposable kit is suitable for the GMP-compliant production of ^{68}Ga-DOTATATE and ^{68}Ga-DOTANOC using commercial generators eluted with 0.05 to 1.0 M HCl. This study represents the first fully automated ^{68}Ga-labelling on the GE TRACERlab® MX module. Further investigations using different peptides are currently ongoing in our laboratories.

References [1] S. R. Banerjee, M. G. Pomper, *Appl. Radiat. Isotop.* **2013**, *76*, 2–13. [2] S. Boschi, F. Lodi, C. Malizia, G. Cicoria, M. Marengo, *Appl. Radiat. Isotop.* **2013**, *76*, 38–45. [3] R. Martin, S. Juettler, M. Mueller, H.-J. Wester, *Nucl. Med. Biol.* **2014**, *41*, 84–89. [4] K. E. Knott, D. Grätz, S. Hübner, S. Jüttler, C. Zankl, M. Müller, *J. Lab. Comp. Radiopharm.* **2011**, *54*, 749–753.