Separation of nca ^{77}As from reactor irradiated GeO_2 targets via liquid-liquid extraction and subsequent labeling of monoclonal antibodies.

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Introduction: Arsenic labeled radiopharmaceuticals could be a valuable source for Positron Emission Tomography (PET) due to their favourable decay characteristics [1,2]. In addition, ^{77}As is an isotope suitable for endoradiotherapy, when high neutron flux reactors are available for its production. This work describes the separation of nca ^{77}As from reactor irradiated GeO_2 targets via liquid-liquid extraction, HBr treatment and subsequent labeling of monoclonal antibodies.

Methods: Reactor produced ^{77}As ($T_{1/2}$ = 38.8 h) was used. All nuclear reactions were performed on natural germanium dioxide targets. The production of ^{77}As was carried out by irradiation of 100-200 mg germanium dioxide for 6 h at the TRIGA Mark II reactor Mainz. Irradiated GeO_2 was placed into a 15 ml plastic vial and 500 µl HF_{conc} was added. After full dissolution, 1.5 ml of HBr_{conc} was added to reduce the arsenic.
Extraction was performed using 500 µl of CCl_4 under vigorous shacking. The organic phase was separated and the procedure was repeated once. The organic fractions were combined and shacked again for about 1 min under the addition of 500 µl PBS-buffer solution (0.5 M hydroxylamine, 25 mM EDTA) to transfer the radioactive arsenic into the aqueous phase. For the determination of the decontamination factor and the overall yields γ-spectroscopy was applied. The organic and aqueous phases were spotted on TLC plates for determination of oxidation state of arsenic [3] (Figure 1.).
Radioactive arsenic isotopes have the possibility of two oxidation states during radiochemical processing: As(III) and As(V). Next to radiochemical purity, the oxidation state is the most important parameter for a successful labeling. Especially for the development of a new separation method, the oxidation state of the arsenic at every state of the process and notably in the final labeling solution is critical.
Labeling was performed with the monoclonal antibody bevacizumab (Avastin®). Tris(2-carboxyethyl)phosphine (TCEP) was utilized to form free SH-groups in the antibody through reduction of disulfide bonds. Therefore, 1.25 mg of Avastin in 50 µl PBS was added directly to a ^{77}As solution in PBS. 10 µl of a TCEP solution (12 mg/ml, 410 nmol) were added and the mixture was incubated at room temperature for 1 hour. Aliquots were taken at various time points and analysed by TLC and HPLC.

Results: The new separation method delivers about 90% of ^{77}As(III) in PBS buffer. The overall yields related to the GeO_2 target were at ≥50%. The purity was 99.98%.
Labeling yields of the mAb of more than 90% could be obtained at room temperature within one hour and under addition of TCEP. An excellent radiochemical purity of the labeled antibody was proven via HPLC, where no free ^{77}As was detectable.

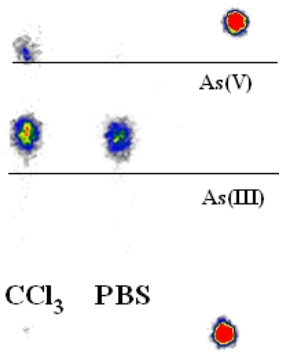

Figure 1: Speciation of As(III) and As(V) via TLC

Conclusions: A very fast method for the separation of radioactive arsenic isotopes from reactor irradiated GeO_2 targets has been developed. To our best knowledge, this method is the fastest procedure published so far. The new method might open the way for routine ^{77}As-labeling of antibodies and subsequent *in vitro* and *in vivo* experiments. The whole procedure can be done in about 15 min and requires only minimal instrumental set-up. Although it was not studied here, the method can be easily transferred to cyclotron produced arsenic isotopes such as ^{72}As or ^{74}As. Additionally, the short time makes this method an ideal candidate for the separation of the short lived ^{70}As ($T_{1/2}$ = 53 min) from cyclotron irradiated targets. The method, however, is not suitable for the use of metallic germanium targets, which do not dissolve in HF_{conc} and where the addition of oxidising agents hinders the extraction of *As(III) from the solution.

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