Development of an alternative method for separation and purification of no-carrier-added arsenic-77 from bulk amounts of germanium-77.

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Introduction: The element arsenic is well known as the favorite poison of the Savellis, the Borgias and Agatha Christie [1]. Another application of arsenic radioisotopes might be its use as radioactive probe in sub-toxic trace amounts for biological or medical purposes. The tracer concept of radiopharmaceutical chemistry allows the application of no-carrier-added (n.c.a.) amounts of radioactive isotopes (e.g. As-77) that are used for labeling of interesting biological carriers like monoclonal antibodies (mAb) and the imaging of their biological behavior *in vivo*.

Experimental:
Dissolving the target: The germanium metal target (100-200 mg) was dissolved in 2 ml of a 1:1-mixture of HF (48%) and hydrogen peroxide (30%). The dissolution was performed in a 15 ml plastic vial with a not tightly screwed cap at RT for about 30 min..
Removing of hydrogen peroxide: After complete dissolution of the target, 5 mg of platinum on activated charcoal (Pt/C, 10%) was given to solution. As a result, degradation of the residual hydrogen peroxide occured. Full removal of H_2O_2 is important, because even trace amounts can change ion exchange behaviour of radioarsenic in the following purification step. Solid particles of platinum on activated charcoal were removed by careful filtration over 30 min.
Anion exchange: 1,8 ml of solution was transferred onto an anion exchange column (0,9 x 6,5 cm, AG 1X8, 200 - 400 mesh) in the chloride form. Elution was performed using 1 ml fractions of a 1:1-mixture of 2M HF and 10M HCl solution. After fraction five was eluted, the eluent was changed to 7 ml 10M HCl and afterwards the column was washed with 5 ml 0,1M HCl to remove impurities of Ge-77 and Ga-72. More then 90% of the ^{77}As(V) was eluted in fractions 10 and 11 (Fig. 1).
Fixing on Bond Elut ENV column and Reduction of As(V) to As(III) with hydroxylamine: Fractions 10 and 11 were combined (2 ml solution) and 2 mg of NaI was added. The solution was passed trough a BondElute column (preconditioned with 5 ml 10M HCl). As a result, ^{77}As was fixed on the resin. ^{77}As(V) was eluted with 200 µl EtOH solution. Reduction of As(V) to As(III) was performed by addition of 500 µl PBS-buffer containing 25 mM EDTA and 0,5M hydroxylamine. As a result more then 95% of arsenic were reduced to +III oxidation state.
Labeling of Bevacizumab: 500 µl of the purified radioarsenic solution in the PBS fraction was combined with 500 µl of Bevacizumab solution (1.25 mg, 8 nmol). 10 µl of TCEP (10 mg/ml, 420 nmol) were added and the mixture was allowed to stand at RT for 1h.

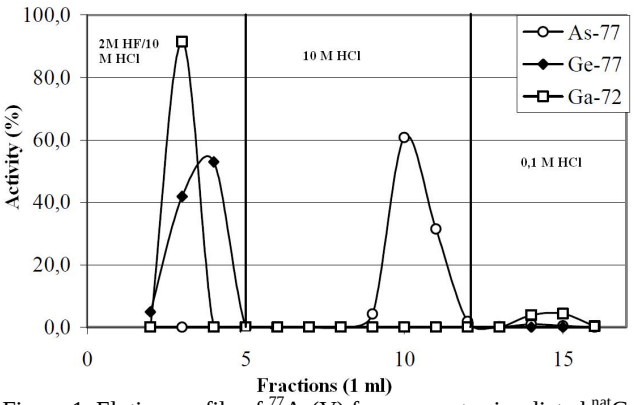

Figure 1. Elution profile of ^{77}As(V) from a reactor irradiated ^{nat}Ge-target on the anion exchange resin AG1X8.

Results: The major advantage of the new separation method is an almost twofold higher yield of 70% in comparison to the previous distillation method with only 40%. The overall separation for germanium/arsenic was $> 1 \cdot 10^6$.
A labeling test of antibodies was successfully exemplified with the monoclonal antibody Bevacizumab. The radiolabeling providing yields of ≥ 99% within 1h incubation time at RT.
One disadvantage of this method is the use of concentrated hydrofluoric acid solution in the dissolution of target and in the subsequent elution of ^{77}Ge from the anion exchanger. To reduce harmful risks the whole procedure and handling ask for extreme care and the use of a protective glove box.

References
[1] Zhu, J., et al., *Nat. Rev. Cancer* **2002**, 2 (9), 705-13.

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