



ELSEVIER

Contents lists available at ScienceDirect

## Applied Radiation and Isotopes

journal homepage: [www.elsevier.com/locate/apradiso](http://www.elsevier.com/locate/apradiso)

# Post-elution processing of $^{44}\text{Ti}/^{44}\text{Sc}$ generator-derived $^{44}\text{Sc}$ for clinical application

M. Pruszyński<sup>a</sup>, N.S. Loktionova<sup>b</sup>, D.V. Filosofov<sup>c</sup>, F. Rösch<sup>b,\*</sup>

<sup>a</sup> Institute of Nuclear Chemistry and Technology, 03-195 Warszawa, Poland

<sup>b</sup> Institute of Nuclear Chemistry, Johannes Gutenberg-University of Mainz, D-55128 Mainz, Germany

<sup>c</sup> Joint Institute of Nuclear Research, DLNP, 141980 Dubna, Russian Federation

## ARTICLE INFO

### Article history:

Received 23 September 2009

Received in revised form

3 March 2010

Accepted 5 April 2010

### Keywords:

$^{44}\text{Ti}$

$^{44}\text{Sc}$

Radionuclide generator

Post-processing of eluates

Cation exchange chromatography

PET/CT

## ABSTRACT

The  $^{44}\text{Ti}/^{44}\text{Sc}$  ( $T_{1/2}$   $^{44}\text{Ti}$ =60 a) generator provides cyclotron-independent access to positron-emitting  $^{44}\text{Sc}$  ( $T_{1/2}$ =3.97 d) for PET imaging. This work aims to post-elution processing of initial  $^{44}\text{Sc}$  generator eluates in order to reduce its volume, HCl concentration and remove the oxalate anions. The on-line adsorption of  $^{44}\text{Sc}$  on cationic resin AG 50W-X8 (200–400 mesh, H<sup>+</sup>-form) is achieved with > 98% efficacy. Subsequently, the purified  $^{44}\text{Sc}$  is desorbed by using 3 ml of 0.25 M ammonium acetate (pH=4.0).

The post-processing takes 10 min. The overall yield of the post-processing reached 90%, which is referred to the  $^{44}\text{Sc}$  obtained from the  $^{44}\text{Ti}/^{44}\text{Sc}$  generator. In addition to the chemical purification, the content of  $^{44}\text{Ti}$  breakthrough was further reduced by one order of magnitude. The 185 MBq generator finally provides 150 MBq of  $^{44}\text{Sc}$  containing < 10 Bq of  $^{44}\text{Ti}$  ready for labeling.

© 2010 Elsevier Ltd. All rights reserved.

## 1. Introduction

Positron emission tomography (PET) is one of the rapidly developing molecular imaging technologies. The commonly used positron emitting radionuclide is  $^{18}\text{F}$  ( $T_{1/2}$ =110 min), and  $^{18}\text{F}$ -labeled fluorodeoxyglucose is the dominating PET tracer.

Another option to support hospitals with relevant PET radionuclides is using radionuclide generators. The decay of a long-lived parent nuclide to a short-lived PET daughter provides an inexpensive and convenient alternative (Rösch and Knapp, 2003). Recently, the  $^{68}\text{Ge}/^{68}\text{Ga}$  generator is turning into an important source of new  $^{68}\text{Ga}$ -labeled radiopharmaceuticals for routine clinical use. The generator provides  $^{68}\text{Ga}$  ( $T_{1/2}$ =67.7 min) from the long-lived  $^{68}\text{Ge}$  ( $T_{1/2}$ =270.8 d). While the short half-life of  $^{68}\text{Ga}$  permits application of suitable  $^{68}\text{Ga}$  activities maintaining an acceptable radiation dose to the patient, it limits application of  $^{68}\text{Ga}$ -labeled tracers to the investigation of fast biological processes.

In this context, generator-derived positron emitters with longer physical half-life are needed—such as  $^{72}\text{As}$  ( $T_{1/2}$ =26 h) from the  $^{72}\text{Se}/^{72}\text{As}$  generator, or  $^{44}\text{Sc}$  ( $T_{1/2}$ =3.97 h) from the  $^{44}\text{Ti}/^{44}\text{Sc}$  generator (Rösch and Knapp, 2003). Hosain et al. (1977)

has already proposed  $^{44}\text{Sc}$  as a PET radionuclide for studying bone disease.  $^{44}\text{Sc}$  is a positron emitter with  $\beta^+$  branching (94.3%), and 99.9% photon emission of 1157.0 keV, generated by  $^{44}\text{Ti}$ . The half-life of  $^{44}\text{Ti}$  was measured by several groups with results varying from 46.4 to 66.6 years. The most recent studies revealed a half-life of  $59.2 \pm 0.6$  years (Ahmad et al., 1998).

The trivalent metal Sc(III) is particularly relevant, as it may be used for labeling of radiopharmaceuticals based on bifunctional chelators, established for coordinating lanthanides such as stable Gd(III) or  $^{177}\text{Lu}$ , as well as for radioactive rare earth metals such as  $^{90}\text{Y}$ , or radionuclides like  $^{111}\text{In}$  and  $^{68}\text{Ga}$ . Due to the increasing medical applications of trivalent radiometals in diagnosis and therapy, the  $^{44}\text{Ti}/^{44}\text{Sc}$  generator could possibly provide an interesting route for PET-imaging using  $^{44}\text{Sc}$  labeled tracers. As a  $\beta^+$  emitter, it could be applied for planning and dosimetric calculations in endoradiotherapy based on the therapeutic radionuclides previously mentioned, but also for matching  $\beta^-$  emitting  $^{47}\text{Sc}$  radiopharmaceuticals (Mausner et al., 1995).

Several strategies were used to design a  $^{44}\text{Ti}/^{44}\text{Sc}$  generator (Greene and Hillman, 1967; Mirza and Aziz, 1969; Seidl and Lieser, 1973; Schumann et al., 2007). The most recent studies on a  $^{44}\text{Ti}/^{44}\text{Sc}$  generator described the concept and experimental parameters of a 185 MBq (5 mCi) generator system, utilizing the anion-exchange resin Bio-Rad AG 1-X8 (200–400 mesh, Cl<sup>-</sup>-form) (Filosofov et al., 2010).  $^{44}\text{Sc}$  was eluted with 20 mL of a 0.005 M H<sub>2</sub>C<sub>2</sub>O<sub>4</sub>/0.07 M HCl mixture and achieved > 97% elution efficacy

\* Corresponding author. Tel.: +49 6131 3925302; fax: +49 6131 3924692.  
E-mail address: frank.roesch@uni-mainz.de (F. Rösch).

for  $^{44}\text{Sc}$ . The breakthrough of  $^{44}\text{Ti}$ , which is the ratio (percentage) of  $^{44}\text{Ti}$  eluted referred to  $^{44}\text{Ti}$  on the generator column, was as low as  $5 \times 10^{-5}\%$ . Because of its large volume (20 mL) and relatively high concentration (0.07 M) of hydrochloric acid, the obtained  $^{44}\text{Sc}$  fraction, however, appears not suitable for direct radio-pharmaceutical syntheses. In many cases, it may prevent a fast, reliable and quantitative labeling procedure.

Consequently, the aim of this work was to develop an efficient and simple method to concentrate and purify the  $^{44}\text{Sc}$  generator eluates adequate for clinical application. Since reducing of volume of  $^{44}\text{Sc}$  in the generator eluate by evaporation of the eluent was inadequate, more sophisticated concentration and purification steps were necessary. A strategy for such an approach relayed on the direct transfer of the initial generator eluate through a cation-exchanger. A similar approach of on-line post-processing of generator eluates was introduced recently for  $^{68}\text{Ge}/^{68}\text{Ga}$  radionuclide generators and proved to be relevant for a versatile labeling strategy (Zhernosekov et al., 2007).

## 2. Materials and methods

### 2.1. Chemicals and reagents

Oxalic acid and ammonium acetate of analytical reagent grade were obtained from Merck (Darmstadt, Germany). All other chemicals were pure reagent grade and used as received unless otherwise specified. The ion exchange resins AG 50W-X4 (200–400 mesh,  $\text{H}^+$ -form), AG 50W-X8 (200–400 mesh,  $\text{H}^+$ -form) and Chelex 100 (200–400 mesh,  $\text{Na}^+$ -form) were purchased at Bio-Rad Laboratories (Richmond, CA, USA). 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) conjugated with D-Phe<sup>1</sup>-Tyr<sup>3</sup>-octreotide (DOTATOC), GMP-grade, was obtained from piChem R&D (Graz, Austria). Deionized Milli-Q water (18.2 M $\Omega$  cm; Millipore) was used in all experiments.

### 2.2. The $^{44}\text{Ti}/^{44}\text{Sc}$ radionuclide generator

The  $^{44}\text{Sc}$  was available from a previously prepared 185 MBq (5 mCi)  $^{44}\text{Ti}/^{44}\text{Sc}$  generator (Filosofov et al., 2010). The  $^{44}\text{Sc}$  was eluted with a 20 mL mixture of 0.005 M  $\text{H}_2\text{C}_2\text{O}_4/0.07$  M HCl with a flow rate of 1 mL/min. The elution profile was determined by fractionation and measuring of  $^{44}\text{Sc}$  and  $^{44}\text{Ti}$  activities in each successive fraction of the eluate.

Measurement of  $^{44}\text{Sc}$  radioactivities was accomplished in a dose calibrator on the curiemeter M2316 (Messelektronik Dresden GmbH, Germany). The  $^{18}\text{F}$  setting was used with a calibration factor of 0.7 to quantify  $^{44}\text{Sc}$  on that instrument. The absolute radioactivity of  $^{44}\text{Sc}$  and  $^{44}\text{Ti}$  was measured by  $\gamma$ -spectrometry using a high-purity germanium (HPGe) well-counter detector using 1157.0 (99.9%) and 1499.5 (0.9%) keV  $\gamma$ -lines for both radionuclides being in decay equilibrium. Measurements of  $^{44}\text{Ti}$  breakthrough radioactivity were performed at least 120 h more than 30 half-lives of  $^{44}\text{Sc}$  after the elution and the  $^{44}\text{Sc}$  measurements.  $^{44}\text{Ti}$  radioactivity was measured also at specific photon emissions of 67.9 and 78.3 keV. All radioactive materials were handled according to approved protocols at the Institute of Nuclear Chemistry at Johannes Gutenberg University Mainz.

### 2.3. Concentration and purification of $^{44}\text{Sc}$ eluate using cation-exchange resin

Post-elution processing studies on  $^{44}\text{Sc}$  eluate obtained from the generator were arranged into three main steps. The first step was focused on adsorbing  $^{44}\text{Sc}$  from a 0.005 M  $\text{H}_2\text{C}_2\text{O}_4/0.07$  M HCl

solution on different ion exchange resins, based on  $K_d$  values of  $^{44}\text{Sc}$  and  $^{44}\text{Ti}$  (Filosofov et al., 2010).

Several strong acidic cation-exchange resins were investigated: AG 50W-X4 (200–400 mesh,  $\text{H}^+$ -form), AG 50W-X8 (200–400 mesh,  $\text{H}^+$ -form) and Chelex 100 (200–400 mesh,  $\text{Na}^+$ -form). Resins were suspended in 1 M NaOH for 15 min, washed several times with Milli-Q water, and re-suspended in 1 M HCl and also washed with Milli-Q water. After this procedure, resins were centrifuged and kept under Milli-Q water for further use.

Small plastic syringes were used to prepare miniaturized chromatography columns, which were plugged with polyethylene filters and packed with different amounts of wet resins. Just before use, the packed columns were conditioned by washing with 1 mL of 4 M HCl and 1 mL Milli-Q water. The  $^{44}\text{Ti}/^{44}\text{Sc}$  generator was eluted according to the previously described protocol with a 20 mL mixture of 0.005 M  $\text{H}_2\text{C}_2\text{O}_4/0.07$  M HCl (Filosofov et al., 2010). Using aliquots of this eluate, the retention of the  $^{44}\text{Sc}$  on different cation-exchangers was checked by passing 2.55–3.0 mL of the  $^{44}\text{Sc}$  solution through pre-treated columns at a flow rate of approximately 1 mL/min at room temperature.

In the second step, different eluate systems were investigated to desorb  $^{44}\text{Sc}$  from the optimum cation-exchange resin identified. Miniaturized chromatography columns were prepared by filling small syringes with 80 mg of wet resin AG 50W-X8 (200–400 mesh,  $\text{H}^+$ -form). The  $^{44}\text{Ti}/^{44}\text{Sc}$  generator was eluted and  $^{44}\text{Sc}$  was adsorbed according to procedures described above. The columns were dried by passing air through them to remove the rest of the generator eluate. Then, columns were washed by 2–4 mL Milli-Q water and dried once again. Next, several solutions at various volumes and concentrations were used to elute  $^{44}\text{Sc}$  from the columns.

In the third step, results obtained from the experiments described above were used to on-line adsorb  $^{44}\text{Sc}$  from the generator eluate. A miniaturized chromatography column (~2 mm inner diameter, ~5 mm length) was prepared using two 3-way valves (Cole-Palmer Instrument Co., Vernon Hills, IL, USA) filled with 53 mg of wet AG 50W-X8 (200–400 mesh,  $\text{H}^+$ -form). The  $^{44}\text{Ti}/^{44}\text{Sc}$  radionuclide generator was connected to the first valve via tubing. The second valve was connected with two capillary tubings directed to the reacting and waste vials, respectively. The  $^{44}\text{Sc}$  as eluted with a 20 mL mixture of 0.005 M  $\text{H}_2\text{C}_2\text{O}_4/0.07$  M HCl at a flow rate of 1 mL/min was transferred on-line through the miniaturized chromatography column. Subsequently, the column was washed by 2–4 mL Milli-Q water and dried by air. Then, 3 mL of 0.25 M ammonium acetate acidified to pH=4.0 by drop-wise addition of acetic acid were passed slowly (0.7 mL/min) through the column.

The  $^{44}\text{Sc}$  eluate was collected in 11 mL glass vial (Mallinckrodt). Finally, the column was reconditioned with 1 mL of 4 M HCl and 1 mL of Milli-Q water. The aliquots of consecutive fractions were collected and measured according to the activity of  $^{44}\text{Sc}$  and  $^{44}\text{Ti}$  using the dose calibrator and  $\gamma$ -spectroscopy, respectively.

## 3. Results and discussion

### 3.1. Elution characteristics

The  $^{44}\text{Ti}/^{44}\text{Sc}$  generator reliably provided 180 MBq of  $^{44}\text{Sc}$  with ~90 Bq of  $^{44}\text{Ti}$  breakthrough during a 1 year period. The  $^{44}\text{Ti}$  breakthrough with respect to the eluted  $^{44}\text{Sc}$  activity was found to be  $5 \times 10^{-5}\%$ . The initial  $^{44}\text{Sc}$  elution profile and the  $^{44}\text{Ti}$  breakthrough are presented in Fig. 1. The highest percentage of  $^{44}\text{Sc}$  was eluted in fractions no. 4–7 (8–14 mL), whereas the fractions no. 1–2 (2–4 mL) contained the highest amount of  $^{44}\text{Ti}$ .

By fractionating the  $^{44}\text{Sc}$  eluate, it is possible to obtain approximately 85% of the available activity in a volume of 8 mL (if only fractions nos. 4–7 are taken) of 0.005 M  $\text{H}_2\text{C}_2\text{O}_4/0.07$  M HCl. This volume appears still too large and the content of hydrochloric acid too high for labeling of, e.g., nanomoles of peptides for application in nuclear medicine. Therefore, an efficient post-elution processing with concentration of  $^{44}\text{Sc}$  eluate on the cation-exchange resin was developed in this study.

### 3.2. Concentration and purification of $^{44}\text{Sc}$

The first step of pre-concentration studies utilized cation-exchange resins to adsorb  $^{44}\text{Sc}$  from the generator eluate of 0.005 M  $\text{H}_2\text{C}_2\text{O}_4/0.07$  M HCl composition. The results of optimization studies concerning the capabilities of different

cation-exchange resins are presented in Table 1. The lowest retention of  $^{44}\text{Sc}$  (42%) was observed when Chelex 100 (200–400 mesh,  $\text{Na}^+$ -form) resin, containing iminodiacetate ions coupled to a styrene divinylbenzene support, was applied. Using the strong cation exchange resin AG 50W-X4 (200–400 mesh,  $\text{H}^+$ -form) with sulfonate groups on the styrene divinylbenzene matrix increased the retention of  $^{44}\text{Sc}$  on the columns up to 51%. Utilization of the AG 50W-X8 (200–400 mesh,  $\text{H}^+$ -form) based on the same matrix with the same functional groups like AG 50W-X4 (200–400 mesh,  $\text{H}^+$ -form), but differing in the cross-linkages value, resulted in 89% retention of  $^{44}\text{Sc}$  on the resin. Therefore, this resin was chosen for the next studies.

In the second step, several miniaturized columns were prepared and filled with 80 mg of the AG 50W-X8 (200–400 mesh,  $\text{H}^+$ -form) resin. Between 2.55 and 3.0 mL of the  $^{44}\text{Sc}$  initial generator eluate was passed through the columns and >98% ( $n=80$ ) of  $^{44}\text{Sc}$  was adsorbed. An additional purification with 2–4 mL Milli-Q water causes some loss of  $^{44}\text{Sc}$  activity (<0.2%).

Then, several solutions were tested for the elution of  $^{44}\text{Sc}$  from the column, c.f. Table 2. Addition of a water-mixable organic solvent promotes chloride complex formation of metal cations in the “outer” coordination sphere. Following a procedure of post-processing  $^{68}\text{Ge}/^{68}\text{Ga}$  generator eluates (Zhernosekov et al., 2007) different mixtures of acetone (90–98%) and HCl (0.05–1.0 M) were tested initially to elute  $^{44}\text{Sc}$  from the cation exchange columns. The obtained  $^{44}\text{Sc}$  recovery was, however, very low (less than 1%), which is in good agreement with literature data on distribution coefficients of  $^{44}\text{Sc}$  at these conditions (Strelow et al., 1971). It seems that only increasing the HCl concentration allows to desorb  $^{44}\text{Sc}$  from the cation-exchange resin. Addition of acetone even at higher percentages did not change the general tendency that formation of Sc(III) complexes with chlorides appears at higher concentration of HCl (more than 1 M) (Hart, 1987).

Elution by 1 mL of 4 M HCl or 0.1 M NaOH resulted in higher recovery of  $^{44}\text{Sc}$ , i.e. 38% and 55%, respectively. Increasing concentration of acid or base or extending the eluting volume further raised the recovery yield. On the other hand, the obtained final solution will be useless for medical application, because of very low or high pH.

Therefore, solutions containing organic complexing anions were used to remove  $^{44}\text{Sc}$  from the cation-exchange resin. Application of 1 mL 0.1 M ethylenediaminetetraacetic acid (EDTA) resulted in 88% recovery of  $^{44}\text{Sc}$ . EDTA forms strong complexes with  $\text{Sc}^{3+}$  ions ( $\log K=21.84$ ) (Perrin, 1979). It was used, e.g. for eluting  $^{90}\text{Y}$  from the  $^{90}\text{Sr}/^{90}\text{Y}$  generator or  $^{68}\text{Ga}$  from the  $^{68}\text{Ge}/^{68}\text{Ga}$  generator (Mikheev et al., 1975; Hnatowich, 1975). The Me(III)-EDTA complexes, however, had to be destroyed prior to labeling reactions, e.g. by heating in the presence of concentrated acids, because EDTA strongly competed with other ligands for labeling (Skraba et al., 1978; Chinol and Hnatowich, 1987).

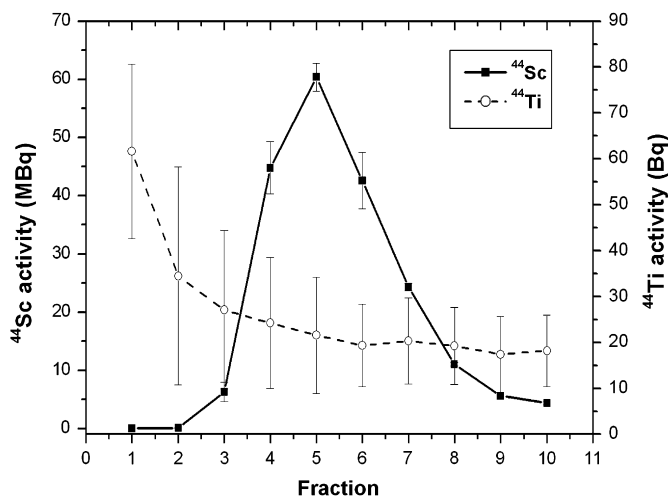


Fig. 1. Elution profile of the  $^{44}\text{Ti}/^{44}\text{Sc}$  generator. One fraction is 2 mL, giving a total eluted volume 20 mL of 0.005 M  $\text{H}_2\text{C}_2\text{O}_4/0.07$  M HCl solution.

Table 1

Retention of the  $^{44}\text{Sc}$  on different cation-exchange resins used in the  $^{44}\text{Sc}$  pre-concentration and purification studies.

Resin	Amount (mg)	Retention of $^{44}\text{Sc}$ (%)
AG 50W-X4	50.0	50.5
AG 50W-X4	160.0	98.0
AG 50W-X8	50.0	88.7
AG 50W-X8	80.0	96.0
AG 50W-X8	200.0	99.9
Chelex 100	51.0	42.2

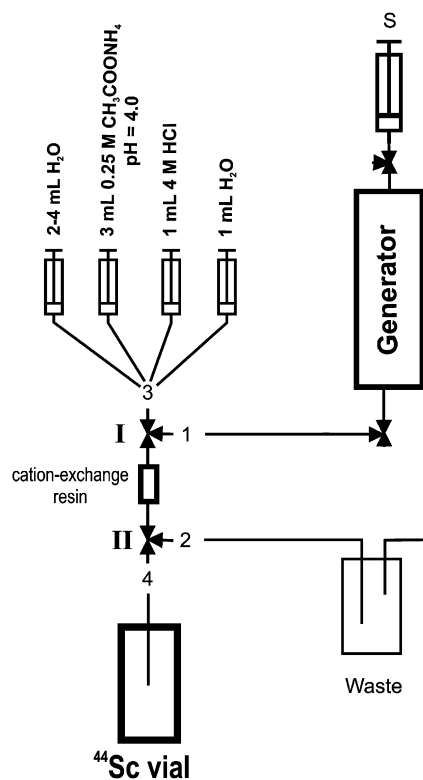
Table 2

Recovery of  $^{44}\text{Sc}$  from miniaturized chromatography columns (80 mg of Bio-Rad AG 50WX-8, 200–400 mesh,  $\text{H}^+$ -form) washed by different solutions.

No.	Solution	Volume (mL)	Recovery of $^{44}\text{Sc}$ (%)
1	Acetone (90–98%)/HCl (0.05–1.0 M)	1.0	< 1.0
2	4.0 M HCl	1.0	37.8
3	0.1 M NaOH	1.0	54.9
4	0.1 M Sodium tartrate	1.0	69.9
5	0.1 M EDTA	1.0	87.6
6	0.1 M Diammonium oxalate	1.0	94.7
7	1.0 M Ammonium acetate	2.0	51.7
8	0.5 M Ammonium acetate/20% EtOH	2.0	45.4
9	0.5 M Ammonium acetate, pH=4.0	2.0	90.4
10	0.25 M Ammonium acetate, pH=4.0	3.0	89.2

**Table 3**Influence of the eluate solution composition on the labeling yield of DOTATOC with  $^{44}\text{Sc}$  and  $^{68}\text{Ga}$ . Labeling conditions: pH=4.0, 95 °C, 15–25 min, 15–40  $\mu\text{g}$  DOTATOC.

Eluate composition	Eluate volume (mL)	Buffer	Nuclide	Yield (%)
0.09 M Diammonium oxalate	1.0	HEPES (4 mL)	$^{44}\text{Sc}$	0.4
97.56% Acetone +0.05 M HCl	0.4	HEPES (4 mL)	$^{68}\text{Ga}$	89.1
97.56% Acetone+0.05 M HCl+0.1 M diammonium oxalate	0.4+1.0	HEPES (4 mL)	$^{68}\text{Ga}$	2.3
0.25 M Ammonium acetate, pH=4.0	3.0	–	$^{44}\text{Sc}$	96.3

**Fig. 2.** Scheme of the post-elution processing of  $^{44}\text{Sc}$ -eluates.

Application of only 1 mL of 0.1 M diammonium oxalate gave the best  $^{44}\text{Sc}$  recovery (95%) compared to the other solutions used (Table 2). However, preliminary studies to label DOTATOC with  $^{44}\text{Sc}$  as eluted by 1 mL of 0.09 M diammonium oxalate and added to 4 mL HEPES (4-(2-hydroxyethyl)piperazine-1-ethane-sulfonic acid) buffer (pH=4.0) containing the peptide, resulted in low synthesis yields of less than 1%. The stability constant of the Sc(III) complex with oxalate anions is quite high ( $\log K_1=7.14$ ) and comparable to Ga(III) ( $\log K_1=6.45$ ) (Gårdhammar, 1971; Smith and Martell, 1976), and at this concentration of about 18 mM oxalates strongly may compete referred to the labeling of nanomoles of the DOTATOC. This hypothesis was later confirmed by labeling DOTATOC with  $^{68}\text{Ga}$  in 4 mL HEPES buffer in the presence of 1 mL of 0.1 M diammonium oxalate at pH=4.0. The labeling reaction yield was around 90% without oxalate solution addition and decreased to  $\sim 2\%$  in the presence of oxalate anions (Table 3).

Therefore, further studies were focused on ammonium acetate solutions, despite the fact that the recovery of  $^{44}\text{Sc}$  by 2 mL of the utilized mixture was slightly lower (Table 2). The stability constant of acetate anions with Sc(III) is much lower ( $\log K=3.48$ ) (Itoh et al., 1984) than with oxalates, so acetates should not compete so strongly in the labeling reaction. Besides, literature

data indicate that acetate solutions are used as buffers in labeling reactions of radionuclides with biomolecules (Hofmann et al., 2001; Bodei et al., 2003; Buchmann et al., 2007). The  $^{44}\text{Sc}$  elution from the miniaturized chromatography columns was optimized according to the composition of acetate solution, concentration and pH. High recovery ( $\sim 90\%$ ) was obtained, when 3 mL of a 0.25 M ammonium acetate buffer acidified to pH=4.0 by addition of acetic acid, was used.

### 3.3. Combined protocol: elution of the $^{44}\text{Ti}/^{44}\text{Sc}$ generator and post-processing in an on-line module

The performed post-elution studies resulted in the building of an on-line module system of generator post-processing (Fig. 2). The key component of the concentration system is a miniaturized chromatography column that was prepared from two 3-way valves (I and II), filled with 53 mg of AG 50W-X8 (200–400 mesh,  $\text{H}^+$ -form) resin and connected with the  $^{44}\text{Ti}/^{44}\text{Sc}$  generator via tubing. The 20 mL of a 0.005 M  $\text{H}_2\text{C}_2\text{O}_4/0.07$  M HCl solution passes the  $^{44}\text{Ti}/^{44}\text{Sc}$  generator with a flow rate of 1 mL/min by using syringe (S) and the eluted  $^{44}\text{Sc}$  adsorbs on-line on the small cationic cartridge (valve I in line 1).

The  $^{44}\text{Sc}$  retains on the column, inbetween the two 3-way valves, whereas the generator eluate continuous to the waste vial (valve II in line 2). Next, valve I is changed to line 3. By using a standard single-use syringe the column with cation-exchange resin is washed by 2–4 mL of  $\text{H}_2\text{O}$  to remove the remaining traces of the initial eluate solution, which are collected in the waste vial as well. Finally, 5 mL air is blown through the column.

After switching the valve II to line 4, the 3 mL of 0.25 M ammonium acetate buffer, pH=4.0, are slowly (0.7 mL/min) pressed through the column with a 2 min break after every 1 mL. Finally, air is passed through the column to remove tracer of the ammonium acetate buffer solution remaining in the dead volume of the column. The  $^{44}\text{Sc}$  is collected in a 11 mL glass reaction vial. Profiles of the  $^{44}\text{Sc}$  and  $^{44}\text{Ti}$  distribution in every step of the post-processing are presented in Table 4. Changing the valve II to line 2 enables reconditioning of the column by washing with 1 mL of 4 M HCl and finally by 1 mL  $\text{H}_2\text{O}$ .

In the first elution step,  $> 98\%$  of cationic  $^{44}\text{Sc}$  was retained on the column, whereas most of the  $^{44}\text{Ti}$  content of the initial generator eluate ( $\sim 80\%$ ) passed the column and was transferred to waste. This is considered as an approach to further remove the amount of co-eluted  $^{44}\text{Ti}$  breakthrough. When the  $\text{H}_2\text{O}$  fraction (2–4 mL) and air were used to remove the excess of the 0.005 M  $\text{H}_2\text{C}_2\text{O}_4/0.07$  M HCl solution remaining on the cation exchanger, elution of both radionuclides was negligible, i.e. less than 0.1% and around 3% for  $^{44}\text{Sc}$  and  $^{44}\text{Ti}$ , respectively. Application of 3 mL ammonium acetate (pH=4.0) recovered  $\sim 90\%$  of  $^{44}\text{Sc}$  without changing the pH of the solution. The amount of  $^{44}\text{Ti}$  in the final  $^{44}\text{Sc}$  fraction was less than 7 Bq. The initial breakthrough of  $5 \times 10^{-5}\%$  was thus further reduced by a factor of 10 reaching a  $5 \times 10^{-6}\%$  level. Reconditioning steps, i.e. washing the column with 1 mL of 4 M HCl and 1 mL Milli-Q water, consequently, removed the rest of  $^{44}\text{Sc}$  and  $^{44}\text{Ti}$  adsorbed on the column and

**Table 4**  
Profile of  $^{44}\text{Sc}$  and  $^{44}\text{Ti}$  in successive steps of post-elution processing on the miniaturized chromatography column (53 mg of Bio-Rad AG 50WX-8, 200–400 mesh,  $\text{H}^+$ -form).

No.	Step	Eluent	Volume (mL)	Relative distribution (%)	
				$^{44}\text{Sc}$	$^{44}\text{Ti}$
1	Generator elution into waste	0.005 M Oxalic acid/0.07 M HCl	20	1.0	80.2
2	Resin purification	$\text{H}_2\text{O}$	2–4	0.1	2.5
3	$^{44}\text{Sc}$ elution	0.25 M Ammonium acetate pH=4.0	3	88.0	7.6
4	Washing	4 M HCl	1	9.9	8.2
5	Washing	$\text{H}_2\text{O}$	1	0.7	1.5

pre-conditioned the resin for another elution. The obtained  $^{44}\text{Sc}$  solution in the acetate buffer was ready for labeling chemistry.

#### 4. Conclusions

The recently developed  $^{44}\text{Ti}/^{44}\text{Sc}$  generator offers the fundamental requirements for radionuclide generators, namely significant differences in the distribution coefficients of mother and daughter radionuclide on the selected resin (Bio-Rad AG 1-X8, 200–400 mesh,  $\text{Cl}^-$ -form) and eluent system (0.005 M  $\text{H}_2\text{C}_2\text{O}_4/0.07$  M HCl), high yields of  $^{44}\text{Sc}$  elution ( $>97\%$ ) and low  $^{44}\text{Ti}$  breakthrough ( $5 \times 10^{-5}\%$ ). In addition, the radiochemical design and the new process of “reverse” elution strategies result in a significant long-term stability of the  $^{44}\text{Ti}/^{44}\text{Sc}$  generator (Filosofov et al., 2010).

However, the large volume of  $^{44}\text{Sc}$  initially eluted and the chemical composition of the initial generator eluate appeared inadequate in the context of radiopharmaceutical chemistry, such as the labeling of nanomoles of precursors.

Comparable situations have been recently addressed by our approach to on-line post-process  $^{68}\text{Ge}/^{68}\text{Ga}$  generator eluates, which usually are being obtained in  $5 \pm 3$  mL of 0.1–1.0 M HCl eluates (Zhernosekov et al., 2007).

The similar strategy applied to the  $^{44}\text{Ti}/^{44}\text{Sc}$  generator also uses an on-line post-processing based on cation-exchanger purification. The Bio-Rad AG 50W-X8 (200–400 mesh,  $\text{H}^+$ -form) resin showed the best parameters both in terms of on-line adsorbing  $^{44}\text{Sc}$  ( $>98\%$ ) and its quantitative recovery is using of 3 mL of 0.25 M ammonium acetate pH=4.0 system ( $\sim 90\%$ ). The content of  $^{44}\text{Ti}$  co-eluted with  $^{44}\text{Sc}$  from the  $^{44}\text{Ti}/^{44}\text{Sc}$  generator of  $5 \times 10^{-5}\%$  is further being reduced by a factor of 10. The final content of  $^{44}\text{Ti}$  in the 140–160 MBq  $^{44}\text{Sc}$  fraction ready for labeling is thus 7 Bq, representing a very low contamination of around  $<2 \times 10^{-7}$ .

This chemically efficient post-elution processing of generator-produced  $^{44}\text{Sc}$  was adapted to a simple module, which allows a rapid and simultaneous concentration and purification of  $^{44}\text{Sc}$  obtained from generator. The post-elution processing of volumes and impurities is easily compatible with the synthesis of  $^{44}\text{Sc}$ -labeled compounds. Thus, the chemically and radiochemically highly pure  $^{44}\text{Sc}$  fraction of very high specific volume activity of around 50 MBq/mL representing 150 MBq overall activity for the first time may allow systematic research on the development and application of new  $^{44}\text{Sc}$ -labeled compounds. Areas of interest are  $^{44}\text{Sc}(\text{III})$  on complex formation, labeling and radiopharmaceutical chemistry of the positron emitter  $^{44}\text{Sc}$ , molecular imaging of  $^{44}\text{Sc}$ -labeled tracers using PET/CT, eventually investigating even new options of  $^{44}\text{Sc}$  molecular imaging by using new PET/3G camera based on  $\beta^+/\gamma$  decay of radionuclide (Huclier-Markai et al., 2008), and finally potential application of diagnostic  $^{44}\text{Sc}$  tracers

matching therapeutic applications of analogue compounds labeled with e.g.  $^{90}\text{Y}$  or  $^{177}\text{Lu}$ , but also with the  $\beta^-$  emitter  $^{47}\text{Sc}$ .

#### Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (DFG Ro. 985/18), the European Commission (FP 6, ToK, POL-RAD-PHARM, MTKD-CT-2004-509224), COST D38 and COST BM0607 and grant of Polish Ministry of Science and Higher Education, Poland, No 126/N-COST/2008/0.

#### References

- Ahmad, I., Bonino, G., Cini-Castagnoli, G., Fischer, S.M., Kutschera, W., Pau, M., 1998. Three-laboratory measurement of the  $^{44}\text{Ti}$  half-life. *Phys. Rev. Letters* 80, 2550–2553.
- Bodei, L., Cremonesi, M., Zobili, S., Grana, C., Bartolomei, M., Rocca, P., Caracciolo, M., Mäcke, H.R., Chinol, M., Paganelli, G., 2003. Receptor-mediated radionuclide therapy with  $^{90}\text{Y}$ -DOTATOC in association with amino acid infusion: a phase I study. *Eur. J. Nucl. Med.* 30, 207–216.
- Buchmann, I., Henze, M., Engelbrecht, S., Eisenhut, M., Runz, A., Schäfer, M., Schilling, T., Haufe, S., Herrmann, T., Haberkorn, U., 2007. Comparison of  $^{68}\text{Ga}$ -DOTATOC PET and  $^{111}\text{In}$ -DTPAOC (Octreoscan) SPECT in patients with neuroendocrine tumours. *Eur. J. Nucl. Med. Mol. Imaging* 34, 1617–1626.
- Chinol, M., Hnatowich, D.J., 1987. Generator-produced yttrium-90 for radioimmunotherapy. *J. Nucl. Med.* 28, 1465–1470.
- Filosofov, D.V., Loktionova, N.S., Rösch, F., 2010. A  $^{44}\text{Ti}/^{44}\text{Sc}$  radionuclide generator for potential nuclear-medical application of  $^{44}\text{Sc}$ -based PET-radiopharmaceuticals. *Radiochim. Acta* 98, 149–156.
- Gårdhammar, G., 1971. Thermodynamic properties of rare earth complexes VIII. Stability constants for oxalate complexes of Sc(III). *Acta Chem. Scand.* 25, 158–168.
- Greene, M.W., Hillman, M., 1967. A scandium generator. *Int. J. Appl. Radiat. Isot.* 18, 540–541.
- Hart, F.A., 1987. Scandium, yttrium and the lanthanides. In: Wilkinson, G., Gillard, R.D., McLeverly, J.A. (Eds.), *Comprehensive Coordination Chemistry. The Synthesis, Reactions, Properties and Applications of Coordination Compounds*. Vol. 3. Main Group and Early Transition Elements. Pergamon Press, Oxford, pp. 1060–1067.
- Hnatowich, D.J., 1975. A method for the preparation and quality control of  $^{68}\text{Ga}$  radiopharmaceuticals. *J. Nucl. Med.* 16, 764–768.
- Hofmann, M., Maecke, H., Börner, A.R., Weckesser, E., Schöffski, P., Oei, M.L., Schumacher, J., Henze, M., Heppeler, A., Meyer, G.J., Knapp, W.H., 2001. Biokinetics and imaging with the somatostatin receptor PET radioligand  $^{68}\text{Ga}$ -DOTATOC: preliminary data. *Eur. J. Nucl. Med.* 28, 1751–1757.
- Hosain, F., Syed, I.B., Spencer, R.P., 1977. The role of positron emitters in nuclear medicine with special reference to scandium-44. *J. Labelled Compd. Radiopharm.* 13, 272.
- Huclier-Markai, S., Montavon, G., Grambow, B., Faivre-Chauvet, A., Barbet, J., 2008. Scandium-DOTA complexes for a new PET/3G camera for medical applications and radio labeling studies. In: *NRC-7—Seventh International Conference on Nuclear and Radiochemistry*, Budapest, Hungary, 24–29 August, Book of Abstracts, p. 64.
- Itoh, H., Itoh, N., Suzuki, Y., 1984. Stability constants of scandium complexes. I. Monocarboxylate complexes species. *Bull. Chem. Soc. Jpn.* 57, 716–718.
- Mausner, L.F., Joshi, V., Kolsky, K.L., Meinken, G.E., Mease, R.C., Sweet, M.P., Srivastava, S.C., 1995. Evaluation of chelating agents for radioimmunotherapy with scandium-47. *J. Nucl. Med.* 36, 104p.
- Mikheev, N.B., Volkova, N.L., Popovich, V.B., 1975. An yttrium-90 generator. *Radiokhimiya* 16, 653–654.
- Mirza, M.Y., Aziz, A., 1969. A scandium generator. *Radiochim. Acta* 11, 43–44.

- Perrin, D.D. (Ed.), 1979. IUPAC Chemical Data Series—No. 22. Stability Constants of Metal-ion Complexes. Part B. Organic Ligands. Pergamon Press, Oxford.
- Rösch, F., Knapp (Russ), F.F., 2003. Radionuclide generators. In: Rösch, F. (Ed.), Handbook of Nuclear Chemistry. Radiochemistry and Radiopharmaceutical Chemistry in Life Sciences, vol. 4. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 90.
- Schumann, D., Horn, S., Neuhausen, J., 2007. Design of a  $^{44}\text{Ti}/^{44}\text{Sc}$  generator system. Annual Report 2006, Paul Scherrer Institut & Universitaet Bern, January 2007, Villigen, Switzerland, p. 41.
- Seidl, E., Lieser, K.H., 1973. Die Radionuklidgeneratoren  $^{113}\text{Sn}/^{113\text{m}}\text{In}$ ,  $^{68}\text{Ge}/^{68}\text{Ga}$  und  $^{44}\text{Ti}/^{44}\text{Sc}$ . Radiochim. Acta 19, 196–198.
- Skraba, W.J., Arino, H., Kramer, H.H., 1978. A new  $^{90}\text{Sr}/^{90}\text{Y}$  radioisotope generator. Int. J. Appl. Radiat. Isot. 29, 91–96.
- Smith, R.M., Martell, A.E., 1976. Critical stability constants. Other Organic Ligands, vol. 3. Plenum Press, New York, p. 94.
- Strelow, F.W.E., Victor, A.H., Van Zyl, C.R., Eloff, C., 1971. Distribution coefficients and cation exchange behavior of elements in hydrochloric acid–acetone. Anal. Chem. 43, 870–876.
- Zhernosekov, K.P., Filosofov, D.V., Baum, R.P., Aschoff, P., Bihl, H., Razbash, A.A., Jahn, M., Jennewein, M., Rösch, F., 2007. Processing of generator-produced  $^{68}\text{Ga}$  for medical application. J. Nucl. Med. 48, 1741–1748.