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Separation of cyclotron-produced ^{44}Sc from a natural calcium target using a dipentyl pentylphosphonate functionalized extraction resin

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Abstract

Significant interest in ^{44}Sc as a radioactive synthon to label small molecules for positron emission tomography (PET) imaging has been recently observed. Despite the efforts of several research groups, the ideal ^{44}Sc production and separation method remains elusive. Herein, we propose a novel separation method to obtain ^{44}Sc from the proton irradiation of calcium targets based on extraction chromatography, which promises to greatly simplify current production methodologies. Using the commercially available Uranium and Tetravalent Actinides (UTEVA) extraction resin we were able to rapidly (< 20 min) recover > 80% of the activity generated at end of bombardment (EoB) in small ~1 M HCl fractions (400 μL). The chemical purity of the ^{44}Sc eluates was evaluated through chelation with DOTA and DTPA, and by trace metal analysis using microwave induced plasma atomic emission spectrometry. The distribution coefficients (K_d) of Sc(III) and Ca(II) in UTEVA were determined in HCl medium in a range of concentrations from zero to 12.1 M. The ^{44}Sc obtained with our method proved to be suitable for the direct labeling of small biomolecules for PET imaging, with excellent specific activities and radiochemical purity.

Keywords

^{44}Sc ; Scandium-44; PET; Radioisotope production; Radiochemical separation; Radiometal; Radiolabeling

1. Introduction

Due to its favorable nuclear properties and amenable chemistry, ^{44}Sc ($t_{1/2}=3.927$ h, 94.3% β^+ , $E_{\text{max}}=1474$ ke V)¹ has been recently recognized as a radiometal holding great potential for PET applications. In spite of this, only a handful of small molecules have been radiolabeled with ^{44}Sc , and even less tested in a preclinical setting (Hernandez et al., 2014; Koumarianou et al., 2012; Koumarianou et al., 2011; Muller et al., 2013; Pruszyński et al.,

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¹All nuclear physics data in this report come from UC Berkeley's *Table of the Isotopes* and were accessed via <http://ie.lbl.gov/education/isotopes.htm>.

2012). This is a result of the non-optimal current production and separation methods, which have limited the broad availability of this isotope. Currently, ^{44}Sc can be produced from a $^{44}\text{Ti}/^{44}\text{Sc}$ generator (Filosofov et al., 2010) and from biomedical cyclotrons via the $^{44}\text{Ca}(p,n)^{44}\text{Sc}$ route (Hoehr et al., 2014; Krajewski et al., 2013; Muller et al., 2013; Severin et al., 2012a; Severin et al., 2012b). However, several issues plague these methodologies. For instance, the difficult production of the parent isotope (^{44}Ti) together with the required extensive post-elution purification of the ^{44}Sc eluate, limit the applicability of generator-based ^{44}Sc (Pruszyński et al., 2010). On the other hand, cyclotron production provides a more efficient method to produce significantly larger ^{44}Sc activities. Nevertheless, a simple yet efficient separation method to isolate ^{44}Sc from the irradiated calcium target remains inexistent. In this study, we describe a novel separation method to obtain ^{44}Sc from irradiated natural calcium targets using extraction chromatography.

Tri-*n*-butylphosphate (TBP), shown in Fig. 1a, is one of the most common organophosphorus extractants, for which extensive data on distribution coefficients of most metals is available (Braun and Ghersini, 1975). Based on this data the separation of Sc(III) from bulk calcium can be effectively achieved in a TBP–HCl extraction system, since Sc(III) and Ca(II) have more than 1000-fold difference in distribution coefficients when the concentration of the acid is 9 M. Hence, given the striking structural similarities between TBP and dipentyl pentylphosphonate (DP[PP]), shown in Fig. 1b, we hypothesized that separation of radioactive scandium from calcium could be achieved using UTEVA, a commercially available resin functionalized with DP[PP].

Herein, we report the successful production and facile UTEVA-based separation of ^{44}Sc from proton irradiated natural calcium targets with excellent yield and chemical purity, in a chemical form amenable for radiolabeling. Additionally, we determined the distribution coefficients of Sc(III) and Ca(II) between UTEVA and HCl to confirm the optimal conditions for their separation. We compare this novel and simple separation method with those from (Krajewski et al., 2013; Muller et al., 2013; Severin et al., 2012a, 2012b), which are based on the difference in binding affinity of scandium and calcium to the chelating resin Chelex 100, the difference in their distribution coefficients in the extraction resin *N,N,N',N'*-tetra-*n*-octyldiglycolamide (DGA), their difference in solubility, and their difference in distribution coefficients in a hydroxamate-functionalized resin, respectively.

Finally, we believe that the implementation of our simple purification method promise to greatly simplify the cyclotron production of radioactive scandium using metallic calcium targets and allow for its readily automation.

2. Materials and methods

Optima grade HCl comes from Aristar Ultra, VWR, West Chester, PA. Natural calcium ($^{\text{nat}}\text{Ca}$, 99.99%) in dendritic chunks comes from Sigma-Aldrich, St. Louis MO. UTEVA (100–150 μm) resin comes from Eichrom, Lisle IL. The cyclic chelating ligand 1,4,7,10-tetraazacyclodecane-1,4,7,10-tetraacetic acid (DOTA) was purchased from Macrocyclics, Dallas TX. The acyclic chelating ligand diethylenetriamine pentaacetic acid (DTPA) was purchased from Acros Organics, Geel, Belgium. Sodium acetate (NaOAc) was purchased

from Fisher Scientific, Pittsburg PA. Both chelating ligands and NaOAc were dissolved in 18 M Ω -cm deionized (DI) water and mixed with Chelex 100 from Sigma-Aldrich for trace metal purification. Scandium foil (99.9%) was purchased from Alfa Aesar, Ward Hill MA. A 50 ppm multi-element standard for calibration and Agilent's 4200 Microwave Plasma Atomic Emission Spectroscopy (MP-AES) system come from Agilent Technologies, Santa Clara CA.

2.1. Cyclotron targetry and irradiations

The target system is very similar to that described in (Severin et al., 2012a). Briefly, 312 ± 19 mg ($n=11$) of ^{nat}Ca were pressed with a hydraulic press at > 400 kg/cm 2 into an annular ring of 1.26 cm 2 , 2.2 mm deep made of aluminum. A 0.56 mm thick silver disk in direct contact with the pressed calcium separated it from water-jet cooling applied on the backside. A 25 μm molybdenum foil was placed over the irradiated face of the target to protect the cyclotron from vaporized calcium. Irradiations were performed on the UW-Madison PETtrace cyclotron using 16 MeV protons for 1 h with an average current of 25 μA .

2.2. Target yields, separation yields and radionuclidic contaminants

^{44}Sc activities for separation yield quantification were measured with a Capintec CRC-15 (Capintec, Ramsey NJ) dose calibrator using the calibration setting 938 suggested by the manufacturer. However, the actual activity from ^{44}Sc and other radionuclidic impurities was measured from the gamma intensities of 50 μL samples placed at distances with known efficiency calibration from a 60 cm 3 high purity germanium (HPGe) detector (Canberra C1519) (FWHM=2.7 keV @ 1333 keV). Gamma-ray spectrum analysis software package, Maestro-32 MCA Emulator (Ortec, Oak Ridge TN), was used to collect and analyze the gamma-ray spectra. The gamma lines used to determine yields are listed in Table 1. The dead time was always kept below 10% and the acquisition time was set so that the statistical uncertainty from the number of counts per peak was kept below 1%, except for the 373 keV peak from ^{43}Sc , for which the statistical uncertainty was 3%. From the accurate activity value of ^{44}Sc it was confirmed that the Capintec measurement was within 10% of the HPGe result, thus this reading was used for the quantification of the separation yields.

Theoretical activity yields at saturation for ^{43}Sc , ^{44}Sc and $^{44\text{m}}\text{Sc}$ at EoB were calculated using the cross-section data from (Levkovskij, 1991). However, this cross-section data was reduced by 20%, due to incorrect assessment of the excitation functions of the monitor reactions in the original publication (Takacs et al., 2002). The stopping power of protons in calcium was obtained from the SRIM software (Ziegler et al., 2013).

2.3. Radiochemical separation

The ~ 300 mg calcium target was pushed out of the target holder cavity into a 50 mL centrifuge tube, to which 5 to 15 mL of concentrated HCl were added to dissolve the target and maintain the H^+ concentration > 9 M. This solution was then transferred manually to one of the three syringes that act as reservoirs connected to an automated module, similar to the one described in (Siikanen et al., 2012), which contains programmable dual pinch valves that control the access to the reservoirs. The module also includes two radiation detectors made in-house by encapsulating PIN photodiodes (HTV S8746-01, Hamamatsu Photonics,

Japan) coupled with a CsI scintillator crystal. One detector monitors the activity in the syringe with the target solution and the other one the activity in the chromatography column. Before the activity was transferred onto the module, new syringes were connected and then filled with several milliliters of DI water to flush the tubing and remove metal impurities left from previous separations. Then, one of the reservoirs was filled with 5 mL of 10 M HCl for the washing step and another one with 400 μ L of deionized water for the ^{44}Sc release step. A peristaltic pump with a flow rate of 1.1 mL/min sent the solution to a 5 mm diameter column cartridge (SPE 1.5 mL reservoir, Grace Davison Discovery Sciences, Deerfield IL) filled with 52 ± 2 mg of UTEVA resin. This packed resin is previously equilibrated with 1 mL of 10 M HCl manually. We selected ~ 50 mg as the amount of resin for practical reasons such as establishing an appropriate resin bed in the column that permitted maximum contact with the mobile phase. Additionally, when we reduced the amount of resin by half (~ 25 mg, Table 5, row a) a significant drop on the trapping efficiency from 83% to 52% was observed, possibly due to an inefficient equilibration between mobile and stationary phases in the column.

The trap and release sequence was as follows:

1. Load the target solution at varying HCl concentrations (9.1, 10.5, 11 M) to trap ^{44}Sc .
2. Wash the column with 5 mL of 10 M HCl.
3. Elute ^{44}Sc in successive fractions of deionized water. The first 400 μ L fraction, with an approximate $[\text{H}^+]$ of 1 M was used as the ^{44}Sc stock solution for labeling of DOTA and DTPA.

Fig. 2 shows a schematic of the automated module with the numbered arrows indicating the sequence of steps used in the separation. Trace metal analysis was performed on samples from three separation runs dissolving the target in 10 mL of concentrated HCl for a final solution concentration of ~ 10.5 M HCl. Table 2 describes the samples that were analyzed using Agilent's 4200 MP-AES system.

2.4. Determination of K_d values of Sc(III) and Ca(II) in UTEVA and HCl solution at various concentrations

K_d values for Sc(III) and Ca(II) in UTEVA were determined in batch experiments using different concentrations of HCl similar to the method described in (Filosofov et al., 2010). ^{44}Sc dissolved in ~ 1 M HCl from one of the separations was used as a tracer of Sc(III). 26.2 mg of 99.99% ^{nat}Ca were dissolved in 6 M HCl, dried down and re-dissolved in 0.05 M HCl in order to have a concentration of 1 mg/20 μ L. Aliquots were prepared in Eppendorf 1.5 mL vials with 100 mg of UTEVA resin. To all solutions 1 mL of HCl was added, then 20 μ L of the ^{44}Sc tracer solution (12.1 MBq), followed by 20 μ L of the ^{nat}Ca solution (1.0 mg). Each of the vials was shaken at room temperature for 4 h in a shaker set at 500 rpm. 400 μ L of the supernatant was taken from every vial and radioactivity A' was measured on the Capintec. The mass of calcium m' in this fraction was assayed using the MP-AES system. The dimensionless K_d value for ^{44}Sc was calculated from the following equation:

$$K_d = \frac{{}^{44}\text{Sc activity per gram of resin}}{{}^{44}\text{Sc activity per mL of liquid}}$$

$$= \frac{[A - (A'/0.4 \text{ mL})(1.04 \text{ mL})] \cdot (1/0.1 \text{ g})}{[(A'/0.4 \text{ mL})(1.04 \text{ mL})] \cdot (1/1.04 \text{ mL})} = \frac{4A - 10.4A'}{A'}$$

A being the activity of the whole vial and A' being the activity of a 400 μL sample of the solution after the extraction, both decay-corrected to the time when the vials were removed from the shaker.

In the case of calcium, K_d was calculated from the analogous equation:

$$K_d = \frac{\text{Ca mass per gram of resin}}{\text{Ca mass per mL of liquid}}$$

$$= \frac{[1.0 \text{ mg} - (m'/0.4 \text{ mL})(1.04 \text{ mL})] \cdot (1/0.1 \text{ g})}{[(m'/0.4 \text{ mL})(1.04 \text{ mL})] \cdot (1/1.04 \text{ mL})} = \frac{4 - 10.4 m'}{m'}$$

2.5. Reactivity with DOTA and DTPA

The reactivity of a radiometal towards a chelating ligand is an indirect method for quantifying the amount of competing nonradioactive metal impurities in a solution. The reactivity of the separated ${}^{44}\text{Sc}$ was assayed using the macrocycle 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) as well as the acyclic chelating ligand diethylene triamine pentaacetic acid (DTPA) following the method described in (Severin et al., 2012a). First, a stock solution is made by mixing 100 μL ($> 74 \text{ MBq}$) of the ${}^{44}\text{Sc}$ eluate with 1 mL of deionized water and 1 mL of 0.25 M sodium acetate (NaOAc). Fractions of 100 μL ($\sim 3.7 \text{ MBq}$) from this buffered solution are distributed into two sets of 10 1.5 mL Eppendorf vials, each containing 100 μL of an aqueous solution of DOTA or DTPA with a known mass of chelator that ranges from zero to 10 μg . All reaction vials had a pH of 4.5. The DOTA solutions were incubated at 85–95 $^\circ\text{C}$ for 1 h and the DTPA solutions at room temperature for 30 min and then spotted onto aluminum backed silica gel ITLC plates (EMD Chemicals, Gibbstown NJ) for Thin Layer Chromatography (TLC), using 0.25 M ammonium hydroxide as the mobile phase. The activity distribution on the plates was assessed by autoradiography with a Packard Cyclone Phosphor-Plate imaging system.

3. Results and discussion

3.1. Cyclotron targetry and irradiations

The metallic calcium target is able to withstand a current of 25 μA without any noticeable effect in its integrity. The 25 μm molybdenum foil degrader drops the 16 MeV proton beam from the PETtrace cyclotron down to $15.56 \pm 0.04 \text{ MeV}$ according to a SRIM simulation (Ziegler et al., 2013). The exit energy after passing through 2.2 mm of calcium is $3.4 \pm 0.4 \text{ MeV}$ also according to a SRIM simulation. This constitutes a thick target since 4.5 MeV is the threshold energy for the ${}^{44}\text{Ca}(p,n){}^{44}\text{gSc}$ reaction.

3.2. Target yields, separation yields and radionuclidic contaminants

Table 3 lists the experimental activity saturation yields at end of bombardment (EoB) of the scandium isotopes produced after our irradiation setting of 15.56 MeV protons, at 25 μ A for one hour. These experimental yields are in agreement with the saturation yields calculated from the excitation functions published in (Levkovskij, 1991) after a correction of 20% (Takacs et al., 2002) and the yields reported in (Severin et al., 2012a).

From our irradiation setting it is reasonable to expect about 0.79 ± 0.08 GBq of ^{44}Sc in the target. The percentage impurities at EoB and 9 h, the timeframe of maximum uptake for many radiolabeled peptides (Hernandez et al., 2014), are presented in Table 4.

The radioactivity of the product is $> 95\%$ ^{44}Sc at EoB and the radionuclidic purity will remain above 90% up to 9 h after EoB. This level of purity is tolerable for preclinical PET studies and perhaps even human studies if dosimetry studies demonstrate tolerable doses from the longer lived impurities $^{44\text{m}}\text{Sc}$, ^{47}Sc and ^{48}Sc , particularly if they potentially accumulate in bone tissue after detaching from an unstable chelate complex. If the radioactive scandium is completely excreted from the body, then we believe that ^{44}Sc from irradiated natural calcium targets is suitable for clinical applications. Natural calcium targets are in metallic form with a thermal conductivity of 200 W/m K, which is comparable to aluminum, 235 W/m K. Calcium is inexpensive and readily available so thick targets for higher yields are economically feasible and target recycling is unnecessary. However, if a higher radionuclidic purity is needed ($> 99\%$, with the coproduced $^{44\text{m}}\text{Sc}$ representing the remaining 1%), then isotopically enriched ^{44}Ca targets are mandatory. To our knowledge, only two groups have reported the use of these target (Krajewski et al., 2013; Muller et al., 2013), both of them employing encapsulated enriched calcium carbonate, $^{44}\text{CaCO}_3$.

3.3. Radiochemical separation

The activity measured with the Capintec dose calibrator was $< 10\%$ higher than the ^{44}Sc activity measured with the HPGe detector. Hence, this reading was used for the determination of separation yields.

Loading the target at a H^+ concentration of ~ 10.5 M, $80 \pm 4\%$ ($n = 7$) of the original activity (decay corrected) was recovered in 400 μL of deionized water. The ^{44}Sc from this separation setting offered the highest separation yield with a high reactivity with DOTA, as will be shown in Section 3.5, and is highlighted in bold in Table 5. Table 5 contains these and other results, together with the parameters involved in the separation. All activities are decay corrected to EoB.

As it was pointed out in Section 2.3, ~ 50 mg of resin was selected for the optimization experiments since we observed a significant drop in the trapping efficiency, from 83% to 52%, when we reduced the amount of resin by half (~ 25 mg, Table 5, row a) when loading the target solution in a HCl concentration of ~ 9.1 M. Combining the results in Tables 5 and 8 we can see that there is a trade off between the separation efficiency and the reactivity of the radioactive scandium. Loading the target solution onto the resin at a concentration of 10.5 M HCl results in the optimum with both high separation yield and reactivity. The final product from this separation method was further characterized by analyzing it with MP-AES

for trace metal contaminants and by titration with the acyclic ligand DTPA. The results from the MP-AES analysis indicated that the most significant metal impurities are: calcium (2.1 mM), iron (93 μM), zinc (72 μM), nickel (29 μM), aluminum (6.4 μM) and manganese (2.0 μM). These results are summarized in Table 6. The concentrations highlighted in bold correspond to the 400 μL fraction of ^{44}Sc employed in the radiolabeling experiments.

The stability constants ($\log K_{ML}$) between metals and ligands, such as DOTA and DTPA, provide a convenient gauge of the ligand's relative affinity for a specific metal (Wadas et al., 2010). The main contaminants in the final product are calcium, iron, zinc and nickel, the three latter ones being important competitors for DOTA chelation based on the magnitude of their thermodynamic stability constants when bound to this chelator: 29.4, 20.8 and 20.0, respectively (Anderegg et al., 2005; Martell et al., 2004), which are comparable to that of Sc(III), 22.5 (Huclier-Markai et al., 2011). The main impurity calcium, on the other hand, is not a strong competitor for DOTA chelation, since its $\log K_{ML}$ value is 17.2 (Anderegg et al., 2005). A typical production run of 25 $\mu\text{A h}$ generates 0.637 GBq of ^{44}Sc in 400 μL , which corresponds to an activity concentration of 1593 GBq/L. Dividing this by the sum of the concentrations, in $\mu\text{mol/L}$, of the main metallic impurities that behave chemically similar to Sc^{3+} ions in aqueous solution (Fe^{3+} , Zn^{2+} , Ni^{2+}) results in an effective specific activity of 8.2 GBq/ μmol , which can be seen in Section 3.5, has the same order of magnitude of the reactivity between scandium and DOTA, 18.1 ± 6.7 GBq/ μmol .

3.4. Determination of K_d values of Sc(III) and Ca(II) in UTEVA and HCl solution at various concentrations

Fig. 3 shows the distribution coefficients (K_d) between resin and acid of scandium and calcium for HCl concentrations ranging from 0 to 12.1 mol/L. Clearly, this plot confirms our hypothesis that the UTEVA resin and TBP have similar extraction properties with respect to calcium and scandium in hydrochloric acid medium.

Furthermore, the separation was possible in a "trap-and-release" fashion employing ~50 mg of UTEVA packed in a 5 mm diameter column with the solution flowing at 1.1 mL/min thanks to the > 10-fold difference in the distribution coefficients between calcium and scandium at a HCl concentration > 9 M.

3.5. Reactivity with DOTA and DTPA

Thin layer chromatography of the DOTA and DTPA titrations showed retention factors of 0.7 and 1.0, respectively, consistent with the production of the scandium complex since these peaks were not present in the control vial with no chelator. These peaks increased in activity concentration as a greater mass of chelating ligand was added to the reaction vial. Plotting the percentage of chelated ^{44}Sc against mass of chelator, a sigmoid curve is obtained from which the reactivity or effective specific activity is calculated by dividing the activity in each vial over the amount of mass with which 50% of the radioactive scandium is chelated, and then multiplying this value times two. When the solution is loaded onto the UTEVA column at a concentration of ~10.5 M, the reactivity was 18.1 ± 6.7 GBq/ μmol , which was enough for radiolabeling the DOTA conjugated cyclic arginine-glycine-aspartate (RGD) dimer E[c(RGDyK)]₂ (Hernandez et al., 2014) as well as the DTPA derivative

cyclohexyldiethylenetriaminepentaacetic acid ligand (CHX-A''-DTPA) conjugated to an antibody fragment at a nmol scale with > 90% yield. The rest of the results are presented in Table 7.

The Sc-DOTA reactivity is about 3 and 5 times less than reported in (Severin et al., 2012a, 2012b), 54 and 100 GBq/ μ mol, respectively. Nevertheless, it has the same order of magnitude as the one inferred from the pioneer work in (Pruszynski et al., 2010), 7.0 GBq/ μ mol, in which the peptide DOTATOC was successfully labeled to ^{44}Sc from a ^{44}Ti generator with > 98% yield. The Sc-DTPA reactivity is more than two times higher than with DOTA, which renders this ligand or its derivatives as potential labels of targeted agents, such as the FDA-approved ^{90}Y -Ibritumomab tiuxetan, tiuxetan being the DTPA derivative 1B4M-DTPA (Camera et al., 1994). We believe that an explanation for the greater reactivity of our separated ^{44}Sc towards DTPA compared to DOTA is due to the much lower thermodynamic stability constant of Ca-DTPA compared to Ca-DOTA, 10.7 and 17.2, respectively (Anderegg et al., 2005). Hence, the remaining bulk calcium in our isolated product, with ~ 2.1 mM concentration, offers less competition for DTPA occupation by the ^{44}Sc isotopes.

Huclier-Markai, et al. (Huclier-Markai et al., 2011) measured the *in vitro* stability of Sc^{3+} complexes with DOTA, DTPA, NOTA, TETA and EDTA in the presence of hydroxyapatite and rat serum and discovered that the most stable one was Sc-DOTA, followed by Sc-DTPA. However, the same kind of stability has not been determined for scandium complexed with modified versions of DTPA. For instance CHX-A''-DTPA was proven to have a greater *in vivo* stability than DTPA when labeled with ^{90}Y (Camera et al., 1994).

3.6. Comparison of our separation method to previous publications

Comparing the separation chemistry of this work with the ones in (Severin et al., 2012a, 2012b) we found many improvements. First, the UTEVA resin is commercially available unlike the hydroxamate resin, which could potentially introduce deviations in the resin's chemical properties. Second, the dissolved target is directly loaded onto the column without having to adjust the pH, which is a process difficult to automate. Third, the ^{44}Sc radioactivity is eluted at high concentration in 400 μL of deionized water ready for labeling, instead of eluting in 1 mL of 0.1 M HCl. And fourth, $80 \pm 4\%$ of the produced radioactive scandium was separated, compared to the overall separation efficiencies of 53% and 63%, reported in (Severin et al., 2012a, 2012b), respectively.

We believe that our separation method based on the UTEVA resin can also be applied with $^{44}\text{CaCO}_3$ targets in order to get a highly concentrated radioactive product and also to improve the recycling efficiency of the expensive target material. This would be recovered by collecting and then processing the eluate with the bulk calcium after trapping the ^{44}Sc just like (Krajewski et al., 2013) did in his separation method based on Chelex 100, in which they achieved a 60% ^{44}Ca recycling efficiency. They claim to have achieved a separation yield of > 70%, which is slightly lower than our $80 \pm 4\%$ separation yield. However, they elute in three 0.5 mL fractions of 1 M HCl, which means that the activity is at a lower concentration and also in a more acidic medium that will be more difficult to buffer for radiolabeling DOTA or DTPA, compared to the product obtained with our method: 400 μL

of deionized water. Of course, our eluate will also be acidic (~1M) due to the residual H⁺ ions that remain in the UTEVA resin after loading and washing with 10 M HCl. Nevertheless, it is clear that the smaller volume of our product, 400 μL, is much easier to buffer than 1.5 mL of 1 M HCl. Muller et al. 2013 achieved a slightly higher separation efficiency of ~85% after using 50–70 mg of DGA resin and then concentrating the activity using a cation exchange resin DOWEX-50. Their isolated ⁴⁴Sc is in 200–400 μL of 1 M ammonium acetate. Table 8 compares our work with the results from these publications.

4. Conclusions

In conclusion, we have described a rapid and facile method to recover ⁴⁴Sc from proton-irradiated metallic calcium targets with excellent yield and high chemical and radiochemical purities. Additionally, the usage of a single column and minimal washing/elution steps provides a convenient framework for the implementation of automatic separation modules. Even though the focus of this work was to study the isolation of ⁴⁴Sc from natural calcium targets, our procedure is readily applicable to the isolation of ⁴⁴Sc from isotopically enriched ⁴⁴CaCO₃ targets or even liquid targets employing Ca(NO₃)₂ solutions. This separation system coupled to improved cyclotron production approaches might be the long waited answer to make ⁴⁴Sc production feasible for translation into the clinic.

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References

- Anderegg G, Arnaud-Neu F, Delgado R, Felcman J, Popov K. Critical evaluation of stability constants of metal complexes of complexones for biomedical and environmental applications (iupac technical report). *Pure Appl Chem.* 2005; 77:1445–1495.
- Braun, T., Ghersini, G. *Extraction Chromatography*. Elsevier, Scientific Pub. Co; Amsterdam, New York: 1975.
- Camera L, Kinuya S, Garmestani K, Wu C, Brechbiel MW, Pai LH, McMurry TJ, Gansow OA, Pastan I, Paik CH. Evaluation of the serum stability and in vivo biodistribution of CHX-DTPA and other ligands for yttrium labeling of monoclonal antibodies. *J Nucl Med.* 1994; 35:882–889. [PubMed: 8176477]
- Filosofov D, Loktionova N, Rosch F. A Ti-44/Sc-44 radionuclide generator for potential application of Sc-44-based PET-radiopharmaceuticals. *Radiochim Acta.* 2010; 98:149–156.
- Hernandez R, Valdovinos HF, Yang Y, Chakravarty R, Hong H, Barnhart TE, Cai W. Sc-44: an attractive isotope for peptide-based pet imaging. *Mol Pharm.* 2014; 11:2954–2961. [PubMed: 25054618]
- Hoehr C, Oehlke E, Benard F, Lee CJ, Hou X, Badesso B, Ferguson S, Miao Q, Yang H, Buckley K, Hanemaayer V, Zeisler S, Ruth T, Celler A, Schaffer P. (⁴⁴g)Sc production using a water target on a 13 MeV cyclotron. *Nucl Med Biol.* 2014; 41:401–406. [PubMed: 24513279]
- Huclier-Markai S, Sabatie A, Ribet S, Kubicek V, Paris M, Vidaud C, Hermann P, Cutler C. Chemical and biological evaluation of scandium(III)-polyamino-polycarboxylate complexes as potential PET agents and radiopharmaceuticals. *Radiochim Acta.* 2011; 99:653–662.
- Koumariou E, Loktionova N, Fellner M, Roesch F, Thews O, Pawlak D, Archimandritis S, Mikolajczak R. Sc-44-DOTA-BN[2–14]NH₂ in comparison to Ga-68-DOTA-BN[2–14]NH₂ in pre-

- clinical investigation. Is Sc-44 a potential radionuclide for PET? *Appl Radiat Isot.* 2012; 70:2669–2676. [PubMed: 23037921]
- Koumariou E, Pawlak D, Korsak A, Mikolajczak R. Comparison of receptor affinity of natSc-DOTA-TATE versus natGa-DOTA-TATE. *Nucl Med Rev Cent East Eur.* 2011; 14:85–89. [PubMed: 22219148]
- Krajewski S, Cydzik I, Abbas K, Bulgheroni A, Simonelli F, Holzwarth U, Bilewicz A. Cyclotron production of Sc-44 for clinical application. *Radiochim Acta.* 2013; 101:333–338.
- Levkovskij, V. Activation cross-sections by protons and alphas. Moscow: 1991. Activation cross section nuclides of average masses (A=40–100) by protons and alpha-particles with average energies (E=10–50 MeV). Accessed via EXFOR: entries A0510002, A0510005 and A0510004.
- Martell A, Smith R, Motekaitis R. NIST Critically Selected Stability Constants of Metal Complexes Database (2004).
- Muller C, Bunka M, Reber J, Fischer C, Zhernosekov K, Turler A, Schibli R. Promises of cyclotron-produced Sc-44 as a diagnostic match for trivalent beta(–)-emitters: in vitro and in vivo study of a Sc-44-DOTA-Folate conjugate. *J Nucl Med.* 2013; 54:2168–2174. [PubMed: 24198390]
- Pruszyński M, Loktionova N, Filosofov D, Rosch F. Post-elution processing of Ti-44/Sc-44 generator-derived Sc-44 for clinical application. *Appl Radiat Isot.* 2010; 68:1636–1641. [PubMed: 20434351]
- Pruszyński M, Majkowska-Pilip A, Loktionova N, Eppard E, Roesch F. Radiolabeling of DOTATOC with the long-lived positron emitter Sc-44. *Appl Radiat Isot.* 2012; 70:974–979. [PubMed: 22464928]
- Severin G, Engle J, Valdovinos H, Barnhart T, Nickles R. Cyclotron produced Sc-44g from natural calcium. *Appl Radiat Isot.* 2012a; 70:1526–1530. [PubMed: 22728844]
- Severin GW, Gagnon K, Engle JW, Valdovinos HF, Barnhart TE, Lewis JS. 44gSc from metal calcium targets for PET. *AIP Conf Proc.* 2012b; 1509:125–128.
- Siikanen J, Peterson M, Tran TA, Roos P, Ohlsson T, Sandell A. A peristaltic pump driven 89Zr separation module. *AIP Conf Proc.* 2012; 1509:206–210.
- Takacs S, Tarkanyi F, Sonck M, Hermanne A. Investigation of the Mo-nat (p,x)Tc-96 mg nuclear reaction to monitor proton beams: new measurements and consequences on the earlier reported data. *Nucl Instrum Methods Phys Res Sect B: Beam Interact Mater Atoms.* 2002; 198:183–196.
- Wadas TJ, Wong EH, Weisman GR, Anderson CJ. Coordinating radio-metals of copper, gallium, indium, yttrium, and zirconium for PET and SPECT imaging of disease. *Chem Rev.* 2010; 110:2858–2902. [PubMed: 20415480]
- Ziegler JF, Ziegler MD, Biersack JP. SRIM – the stopping and range of ions in matter. 2013

HIGHLIGHTS

- Cyclotron-produced ^{44}Sc is loaded into 50 mg of UTEVA resin after dissolution of 300 mg of $^{\text{nat}}\text{Ca}$ target in concentrated HCl.
- $80 \pm 4\%$ of the activity at end of bombardment (EoB) is eluted using 400 μL of deionized water in < 20 min after EoB.
- The separation factor of the bulk calcium is 1.2×10^4 .
- ^{44}Sc has reactivities or effective specific activities with DOTA and DTPA of 18.1 ± 6.7 and 49 ± 37 GBq/ μmol , respectively.
- Metal impurities in the isolated ^{44}Sc are: Ca (2.1 mM), Fe (93 μM), Zn (72 μM), Ni (29 μM), Al (6.4 μM) and Mn (2.0 μM).

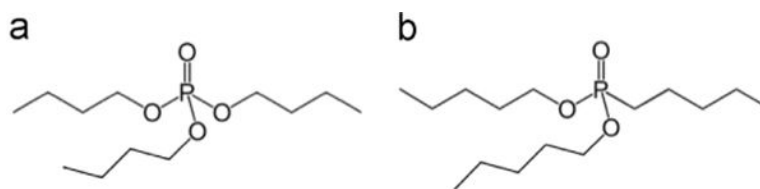


Fig. 1. (a) Tri-n-butylphosphate (TBP) and (b) Dipentyl pentylphosphonate (DP[PP]), the active extractant in the UTEVA resin.

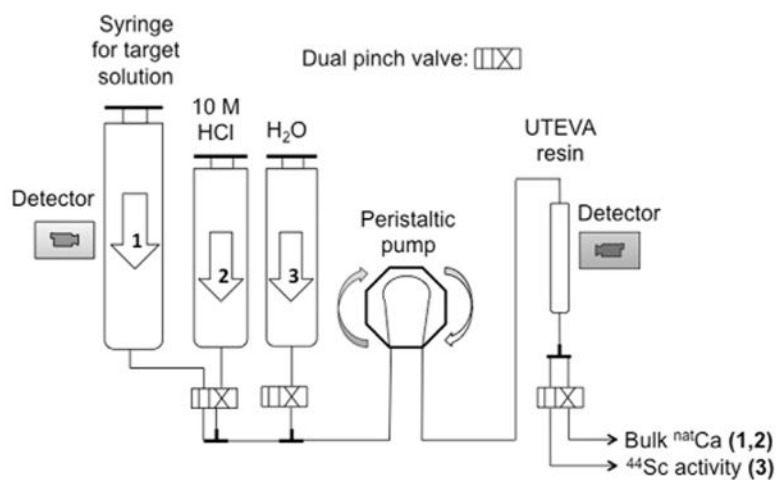


Fig. 2. Scheme of the separation process overlaid in a schematic of the automated module.

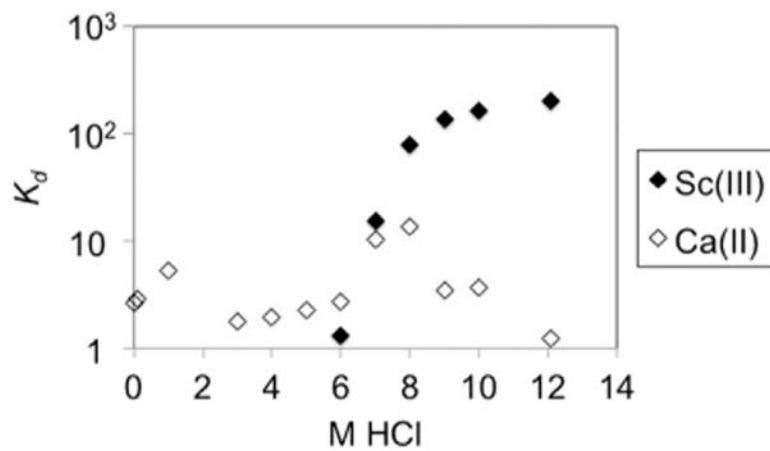


Fig. 3. Distribution coefficients of Sc(III) and Ca(II) in UTEVA resin at different HCl concentrations.

Table 1

Gamma emissions from the scandium radioisotopes used for determining yields.

Nuclide	Gamma energies (keV)	Branching ratio
^{43}Sc	373	0.23
^{44}Sc	1157	0.999
$^{44\text{m}}\text{Sc}$	271	0.867
^{47}Sc	159	0.683
^{48}Sc	983.5	1.00

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Table 2

Samples analyzed by MP-AES.

	Volume (μL)	Sample description
i)	50	Target dissolution in 10 mL
ii)	50	Eluate after trapping Sc
iii)	50	Eluate from 5 mL 10 M HCl wash
iv)	400	First eluted fraction with isolated Sc
v)	200	Second eluted fraction with isolated Sc

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Table 3

Natural abundances of calcium isotopes and their radioactive scandium reaction products after proton bombardment at 15.56 MeV.

Target	% abundance	Nuclear reaction	$t_{1/2}$	Measured saturation yield (MBq/ μ A)	Predicted yield using corrected data in (Levkovskij, 1991) (MBq/ μ A)	% of predicted yield	% of yield measured in (Severin et al., 2012a) at 16 MeV
^{40}Ca	96.941	$^{40}\text{Ca}(p,n)^{40}\text{Sc}^a$	182 ms	–	–	–	–
^{42}Ca	0.647	$^{42}\text{Ca}(p,n)^{42m}\text{Sc}^a$	61.7 s	–	–	–	–
		$^{42}\text{Ca}(p,n)^{42g}\text{Sc}^b$	681 ms	–	–	–	–
^{43}Ca	0.135	$^{43}\text{Ca}(p,n)^{43}\text{Sc}^b$	3.89 h	4.870.8	7.06	68%	90%
		$^{43}\text{Ca}(p,2n)^{42}\text{Sc}^a$	681 ms	–	–	–	–
^{44}Ca	2.086	$^{44}\text{Ca}(p,n)^{44g}\text{Sc}$	3.93 h	179 ± 22	257	70%	84%
		$^{44}\text{Ca}(p,n)^{44m}\text{Sc}$	58.6 h	15.2 ± 2.0	20.0	76%	88%
		$^{44}\text{Ca}(p,2n)^{43}\text{Sc}^b$	3.89 h	–	–	–	–
^{46}Ca	0.004	$^{46}\text{Ca}(p,n)^{46}\text{Sc}$	83.8 h	<i>Unobserved</i>	–	–	75%
^{48}Ca	0.187	$^{48}\text{Ca}(p,n)^{48}\text{Sc}$	43.7 h	15.6 ± 2.4	–	–	129%
		$^{48}\text{Ca}(p,2n)^{47}\text{Sc}$	80.4 h	14.5 ± 2.5	–	–	–

^aThese reactions are practically negligible because of the too short half-life of the produced Sc-isotope.

^bThe ^{43}Sc produced from $^{44}\text{Ca}(p,2n)$, with an energy threshold of 14.5 MeV, is not distinguished from $^{43}\text{Ca}(p,n)$ in this study.

Table 4Radionuclidic purity of the separated scandium at EoB and 8 h after EoB ($n=8$).

Scandium isotope	% of total activity at EoB	% of total activity 9 h after EoB
^{44g}Sc	95.7 ± 0.3	90.6^a
^{44m}Sc	0.6 ± 0.1	2.3
^{43}Sc	2.6 ± 0.3	2.3
^{47}Sc	0.40 ± 0.05	1.7
^{48}Sc	0.8 ± 0.1	3.0

^aBateman's equilibrium equation between ^{44m}Sc and ^{44g}Sc was included in the computation of this value.

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Table 5

Results of separation experiments.

<i>n</i>	Calcium mass (mg)	mL of conc. HCl added for dissolution	Molarity of dissolution ^a (mol/L)	UTEVA mass (mg)	% of activity trapped in UTEVA	% of total activity eluted in 1st fraction (400 μ L DI)	% of total activity eluted in 2nd fraction (200 μ L DI)
a)	295.7	5	~9.1	24.5	52	43	2.5
b)	313 \pm 14	5–5.5	~9.1	52 \pm 1	83 \pm 5	68 \pm 4	4.7 \pm 1.6
c)	317 \pm 22	10	~ 10.5	52 \pm 3	89 \pm 3	80 \pm 4	5.1 \pm 1.3
d)	304 \pm 8	15	~11.1	52 \pm 1	92 \pm 2	82 \pm 2	5.6 \pm 2.7

^aThe molarity is estimated by subtracting the amount of moles of hydrogen released as H₂ gas from the amount of moles of H⁺ ions in solution, and then dividing this by the total volume of the dissolution. The amount of H₂ that is released is estimated from the reaction equation $\text{Ca}+2\text{HCl}\rightarrow\text{CaCl}_2+\text{H}_2$.

Table 6

Results from MP-AES analysis on the samples described in Table 2.

Sample	i)	ii)	iii)	iv)	v)	
Ca^a	(ppm)	32770 ± 932	1110 ± 127	82.2 ± 16.5	57.3 ± 7.4	
	(mM)	818 ± 23	<i>Overrange</i>	27.7 ± 3.2	2.1 ± 0.4	1.4 ± 0.2
	Mass in total volume (mg)	309.6 ± 5.2	<i>Overrange</i>	5.55 ± 0.64	0.027 ± 0.001	0.012 ± 0.002
Separation factor						
Fe	(ppm)	0.6 ± 0.3	0.32 ± 0.09	0.16 ± 0.14	5.2 ± 3.2	1.2 ± 0.8
	(µM)	10 ± 6	5.6 ± 1.5	2.8 ± 2.5	93 ± 58	22 ± 15
	Mass in total volume (µg)	5.6 ± 3.2	5.1 ± 3.6	0.90 ± 0.54	1.8 ± 0.8	0.27 ± 0.21
Separation factor						
Zn	(ppm)	0.7 ± 0.4	1.6 ± 0.8 ^b	2.7 ± 1.1	4.7 ± 1.3	5.8 ± 2.4
	(µM)	10 ± 6	25 ± 12	52 ± 16	72 ± 20	88 ± 36
	Mass in total volume (µg)	6.6 ± 3.9	15.5 ± 7.8	14 ± 5	1.7 ± 0.7	1.2 ± 0.4
Separation factor						
Ni	(ppm)	2.7 ± 1.5	2.8 ± 1.9 ^b	2.7 ± 1.2	1.7 ± 1.3	0.90 ± 0.59
	(µM)	46 ± 26	48 ± 33	47 ± 21	29 ± 22	15 ± 10
	Mass in total volume (µg)	26 ± 18	26 ± 18	10 ± 6	0.58 ± 0.39	0.18 ± 0.17
Separation factor						
Al	(ppm)	9.6 ± 0.1	7.6 ± 0.1	1.2 ± 0.7	0.17 ± 0.02	0.18 ± 0.09
	(µM)	354 ± 1	281 ± 2	44 ± 26	6.4 ± 0.7	6.7 ± 3.4
	Mass in total volume (µg)	91.3 ± 0.4	71.7 ± 0.4	8.4 ± 1.2	0.059 ± 0.017	0.046 ± 0.017
Separation factor						
Mn	(ppm)	0.77 ± 0.30	0.90 ± 0.79 ^b	0.12 ± 0.90	0.11 ± 0.07	0.07 ± 0.06
	(µM)	14 ± 5	16 ± 14	2.2 ± 1.5	2.0 ± 1.3	1.3 ± 1.0
	Mass in total volume (µg)	7.3 ± 2.7	8.4 ± 7.2	0.59 ± 0.43	0.041 ± 0.031	0.016 ± 0.012
Separation factor						
				1559	178	

^aThe calcium content in sample i) was calculated from the measured mass of calcium dismounted from the target system.^bThe increase in Zn, Ni and Mn content in the eluate after loading the Sc may come from the UTEVA resin or the tubing in the separation module.

Table 7

Results of the DOTA-Sc and DTPA-Sc reactivity experiments from the separation runs explained in Table 5.

	Reactivity of 1st fraction with DOTA (GBq/ μ mol)	Reactivity of 1st fraction with DTPA (GBq/ μ mol)
a)	15.2	–
b)	18.5 \pm 3.5	–
c)	18.1 \pm 6.7	49 \pm 37
d)	3.8 \pm 0.7	–

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Table 8

Comparison of the separation methods used for the separation of radioactive Sc from calcium-based targets.

Method	Target	Proton energy (MeV)	Activity yield (MBq/ μ Ah)	Separation yield (%)	Volume of ^{44}Sc eluate (mL)	Matrix of separated ^{44}Sc	Calcium conc. in separated ^{44}Sc (mM)	Specific Activity with DOXA (GBq/ μ mol)	Reference
Filtration	^{44}Ca (99.99%)	16	38 ± 3	40 ± 18	0.15	0.1 M HCl	2.3	54 ± 14	(Severin et al., 2012a)
Hydroxamate resin	^{44}Ca (99.99%)	16	38 ± 3	63 ± 15	1.0	0.1 M HCl	<i>Not reported</i>	50	(Severin et al., 2012b)
Chelex 100	$^{44}\text{CaCO}_3$ (94.53%)	9.0	8.9	70	0.50	1 M HCl	< 0.025	22	(Krajewski et al., 2013)
DGA+DOWEX-50	$^{44}\text{CaCO}_3$ (97.00%)	17.6 ± 1.8	11.7	85	0.20–0.40	1 M NH_4OAc	<i>Not reported</i>	5.2	(Muller et al., 2013)
UTEVA	^{44}Ca (99.99%)	15.56 ± 0.04	32 ± 3	80 ± 4	0.40	~ 1 M HCl	2.1 ± 0.4	18.1 ± 6.7	This work