

Cyclotron Production and Separation of Scandium Radionuclides from Natural Titanium Metal and Titanium Dioxide Targets

C. Shaun Loveless^{1,2}, Jose R. Blanco³, George L. Diehl III⁴, Rawdah T. Elbahrawi⁵, Tommaso S. Carzaniga⁶, Saverio Braccini⁶, Suzanne E. Lapi^{1,3*}

¹Department of Radiology, University of Alabama at Birmingham, Birmingham, AL, 35233

²Department of Chemistry, Washington University in St. Louis, St. Louis, MO, 63134

³Department of Chemistry, University of Alabama at Birmingham, Birmingham, AL, 35233

⁴Department of Chemistry, Vassar College, Poughkeepsie, NY, United States

⁵Department Biomedical Engineering, University of Alabama at Birmingham, Birmingham, AL, 35233

⁶Albert Einstein Center for Fundamental Physics, University of Bern, CH-3012 Bern, Switzerland

*Corresponding author

First Author:

C. Shaun Loveless, 1720 2nd Avenue South, Birmingham, AL, 205-934-6650, cslove@uab.edu, graduate student

Corresponding Author:

Dr. Suzanne E. Lapi, 1720 2nd Avenue South, Birmingham, AL, 205-975-8689, lapi@uab.edu

Running Title: Cyclotron Produced Radioscandium

Abstract

Background

Theranostic strategies, which combine diagnostic and therapeutic agents, involve select radionuclides that allow diagnostic imaging and tailored radionuclide therapy in the same patient. An example of an FDA-approved theranostic pair are the ^{68}Ga and ^{177}Lu labeled DOTATATE peptides, which are used to image neuroendocrine tumors, predict treatment response, and treat disease. However, when using radionuclides of two different elements, differences in the pharmacokinetic and pharmacodynamic profile of the agent can occur. Theranostic agents that incorporate the matched-pair radionuclides of Sc, $^{43}\text{Sc}/^{47}\text{Sc}$ or $^{44}\text{Sc}/^{47}\text{Sc}$, would guarantee identical chemistries and pharmacological profiles. The aim of this study was to investigate production of $^{43,44,47}\text{Sc}$ via proton induced nuclear reactions on Ti nuclei using a 24MeV cyclotron.

Methods

Al, Nb, and Ta target holders were used with Ti foils and pressed TiO_2 to produce Sc radionuclides with proton energies up to 24MeV. Irradiated targets were digested using NH_4HF_2 and HCl in a closed perfluoroalkoxy alkane vessel in 90min. Scandium radionuclides were purified via ion-exchange chromatography using branched N,N,N',N'-tetra-2-ethylhexyldiglycolamide (BDGA). The Ti target material was recovered via alkali-precipitation with ammonia solution.

Results

Titanium foil and TiO_2 were digested with an average efficiency of $(98\pm 3)\%$ and $(95\pm 1)\%$, respectively. The typical digestion time was 45min (Ti foil) and 75min (TiO_2). The average

Sc recovery was $(94\pm 3)\%$ and the average Ti recoveries from digested Ti foil and TiO_2 post-precipitation as TiO_2 were $(108\pm 8)\%$ and $(104\pm 5)\%$ of initial mass, respectively.

Conclusion

This work demonstrated a robust method for the cyclotron production of Sc radionuclides that could be used with natural or enriched TiO_2 target material.

Keywords

^{43}Sc , ^{44}Sc , ^{47}Sc , Ti Digestion, BDGA, Cyclotron Production

Introduction

The element Sc has three radionuclides with properties suitable for diagnostic and therapeutic applications in nuclear medicine. Scandium-43 ($t_{1/2}=3.89\text{h}$) and ^{44}Sc ($t_{1/2}=3.97\text{h}$) undergo positron decay, with β^+ mean energies of 476.0keV (88.1%) and 632.0keV (94.3%), respectively, making them well-suited for positron emission tomography (PET) (1). Scandium-47 ($t_{1/2}=3.35\text{d}$) undergoes beta decay, with a β^- mean energy of 162keV to ^{47}Ti , followed by emission of a 159keV gamma-ray (68.4%) (1). Thus, ^{47}Sc could be used for targeted radionuclide therapy and single-photon emission computed tomography (SPECT). These radionuclides of Sc could be used to create theranostic agents for diagnostic imaging and targeted radiotherapy in the same patient.

Interest in these radionuclides is partly motivated by the recent clinical success of DOTA-(Tyr³)-octreotate (DOTATATE) and results in clinical trials investigating small-molecule inhibitors of prostate specific membrane antigen (PSMA). DOTATATE, a somatostatin analogue, labeled with ^{68}Ga ($t_{1/2}=67.7\text{min}$) or ^{177}Lu ($t_{1/2}=6.7\text{d}$) can be used in theranostics to diagnose and treat SSTR-expressing neuroendocrine tumors (2,3). Similarly, inhibitors of PSMA such as PSMA-11, PSMA-617, DCFPyL and MIP-1072 labeled with ^{68}Ga , ^{177}Lu , ^{18}F ($t_{1/2}=109.7\text{min}$), ^{131}I ($t_{1/2}=8.0\text{d}$), ^{123}I ($t_{1/2}=13.2\text{h}$) or $^{99\text{m}}\text{Tc}$ ($t_{1/2}=6\text{h}$) can be used for diagnosis and treatment of PSMA positive prostate cancer (1,4). Theranostic agents that incorporate radionuclides of different elements can have differences in their in vivo kinetics (5). The selection of a matched-pair (e.g., $^{43/47}\text{Sc}$), guarantees identical pharmacodynamic and pharmacokinetic profiles, and provides the truest picture of in vivo distribution, prior to radionuclide therapy. Further, the half-lives of $^{43}\text{Sc}/^{44}\text{Sc}$, in contrast to ^{68}Ga , could allow for imaging at extended time points (6).

Therefore, development of efficient methods to produce $^{43,44,47}\text{Sc}$ is an important first step to translating these matched-pair radionuclides of Sc to the clinic.

Scandium-43 and ^{44}Sc can be produced via multiple routes. Accelerator production using α particles, protons, and deuterons on Ca and Ti nuclei has been investigated for the $^{\text{nat}}\text{Ca}(\alpha,x)^{43,44,47}\text{Sc}$, $^{42}\text{Ca}(\text{d},\text{n})^{43}\text{Sc}$, $^{43}\text{Ca}(\text{p},\text{n})^{43}\text{Sc}$, $^{44}\text{Ca}(\text{p},2\text{n})^{43}\text{Sc}$, $^{46}\text{Ti}(\text{p},\alpha)^{43}\text{Sc}$, $^{47}\text{Ti}(\text{p},\alpha)^{44}\text{Sc}$, $^{44}\text{Ca}(\text{p},\text{n})^{44}\text{Sc}$, $^{\text{nat}}\text{Ca}(\text{p},\text{n})^{44}\text{Sc}$ and $^{44}\text{Ca}(\text{d},2\text{n})^{44}\text{Sc}$ nuclear reactions (7-13). The use of Ca and Ti have been shown to provide GBqs and hundreds of MBqs of ^{43}Sc and ^{44}Sc depending upon beam current and irradiation time; however, ^{44}Sc produced by these reactions will result in the co-production of its nuclear isomer, $^{44\text{m}}\text{Sc}$ ($t_{1/2}=58.6\text{h}$). The presence of this longer-lived metastable state may complicate patient dosimetry. Scandium-44 may be obtained without its nuclear isomer from a ^{44}Ti ($t_{1/2}=59.2\text{y}$) generator (14).

Scandium-47 can also be produced via multiple routes. Reactor production of ^{47}Sc has been investigated using fast neutrons on enriched Ti via the $^{47}\text{Ti}(\text{n},\text{p})^{47}\text{Sc}$ reaction ($E_{\text{n}}>1\text{MeV}$) or thermal neutrons ($E_{\text{n}}=0.025\text{eV}$) on enriched Ca via the $^{46}\text{Ca}(\text{n},\gamma)^{47}\text{Ca}\rightarrow^{47}\text{Sc}$ reaction for a $^{47}\text{Ca}-^{47}\text{Sc}$ ($t_{1/2}=4.5\text{d}$) generator system (15). One challenge facing development of a $^{47}\text{Ca}-^{47}\text{Sc}$ generator is the natural abundance of ^{46}Ca (0.004%). Scandium-47 may also be produced by the $^{48}\text{Ti}(\gamma,\text{p})^{47}\text{Sc}$ reaction using bremsstrahlung radiation and accelerator production by the $^{48}\text{Ca}(\text{p},2\text{n})^{47}\text{Sc}$ reaction was investigated at 18MeV and resulted in a saturation yield of 12 GBq/ μA (16-19).

Unlike production routes using Ca, the use of Ti could allow production on a single proton accelerator using a unified separation methodology that uses identical targetry and

target processing chemistries, and results in better radionuclidic purity of $^{43,44,47}\text{Sc}$. However, the magnitude of the cross section for reactions on Ti are much less than those on Ca at lower energies (16-18MeV). Favorably, the cross section values increase at the higher energies (up to 24MeV) used in this study. Multiple challenges must be addressed to produce Sc radionuclides from stable nuclei of Ti using this unified approach. First, the powdery TiO_2 targets require a holder that encapsulates the irradiated material to prevent radiological contamination. Next, the slow TiO_2 digestion kinetics and short half-life of $^{43}\text{Sc}/^{44}\text{Sc}$ necessitate development of a rapid digestion method. Lastly, $^{43,44,47}\text{Sc}$ with high radionuclidic purity can only be obtained using enriched Ti, since $^{46,47,50}\text{Ti}$ have natural abundancies of 8.25%, 7.44%, and 5.18%, respectively. Thus, efficient recycling chemistry must be developed to recover and reuse the enriched target material.

To address these challenges, methods were developed to investigate $^{43,44,47}\text{Sc}$ yields and purification chemistry using natural Ti and TiO_2 . The results from this work suggest that $^{43,44,47}\text{Sc}$ could be produced at energies up to 24MeV using enriched TiO_2 and purified using a unified methodology to supply quantities needed for the pre-clinical evaluation of these matched pair radionuclides.

Materials and Methods

Reagents

All chemicals were analytical or trace metals grade. Deionized 18.2M Ω ·cm water (Milli-Q System; Millipore, Billerica, MA) was used unless otherwise specified. Ammonium bifluoride (NH_4HF_2 , 99.999%), hydrochloric acid (HCl, 37% wt., 99.999%), 25% ammonia solution (NH_4OH , EMSURE®), TiO_2 (anatase form, 99.8%), periodic table analytical

standards (Mix 1, 10mg/L, TraceCERT®), ammonium acetate (CH₃CO₂NH₄, 99.999%) and nitric acid (HNO₃, 70% wt., 99.999%) were purchased from Sigma-Aldrich (St. Louis, Missouri). Analytical standards containing Ti (1000mg/L) and Sc (1000mg/L) were purchased from Agilent (Santa Clara, CA). Analytical grade BDGA resin was purchased from Eichrom Technologies (Lisle, IL). 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) was purchased from Macrocyclics (Plano, TX). All glassware was cleaned in a 50% HNO₃ bath overnight.

Target Holders

Multiple target holders were developed to mount and irradiate Ti foil and TiO₂ target materials. The disc shaped 2mm thick target holders fabricated from Al, Ta, and Nb are shown in Figure 1. The Al target holder had a circular-shaped base and cap secured by samarium-cobalt (SmCo) magnets. A fluoroelastomer o-ring was integrated between the base and cap to enclose the target material. A bore ($\varnothing=7\text{mm}$) was centered in the base. The Nb target holder contained a centered bore ($\varnothing=10\text{mm}$) holding Ti foil which was secured against the base using a 10.1mm single-turn spiral internal retaining ring (Smalley, Lake Zurich, IL). The Ta target holder contained a centered bore ($\varnothing=10\text{mm}$) with an integrated o-ring between the base and cap to enclose the target material. The bore held a TiO₂ disc that was secured by placing a 100 μm Ti disc atop the bore and o-ring. The Ti disc was held in place by a 14.3mm spiral retaining ring (Rotor Clip, Somerset, NJ). Stopping and Range of Ions in Matter (SRIM) was used to determine the optimal thickness of each target holder to stop 17 or 24MeV protons (20).

Preparation of Target Material

Titanium foil targets were prepared using a 10mm punch and die (Precision Brand Products, Downers Grove, IL). Prior to pressing, TiO₂ was dried overnight at 230°C in a vacuum oven at 0.3Pa. Approximately 35 or 70mg of TiO₂ was transferred into a clean 7 or 10mm die (Specac™, Kent, United Kingdom) and pressed for 5min at 4tons pressure on a manual press (Carver, Wabash, Indiana). Additional TiO₂ was iteratively added and pressed. This process was repeated twice with a final target masses of approximately 52 or 110mg.

Cyclotron Parameters

Irradiations were conducted on a TR-24 cyclotron (Advanced Cyclotron Systems, Inc., Richmond, BC, Canada). Scandium-43, ⁴⁴Sc, and ⁴⁷Sc were produced via the ^{nat}Ti(p,x) nuclear reaction at 17 and 24MeV. Titanium and TiO₂ targets were mounted at a 90° angle and bombarded at beam currents up to 40 and 30μA, respectively. The targets were cooled by high-pressure, low-flow He gas (275 to 345kPa, 1L/min) on the front and pumped water (2 to 3L/min) on the back. Bombarded targets were allowed to decay for 1 to 5h to allow for the decay of co-produced ⁴⁷V (t_{1/2}=32.6min).

Production Yields

The radionuclidic yields were determined using a high-purity germanium (HPGe) detector. Predicted yields were calculated using nuclear data retrieved from the Experimental Nuclear Reaction Data database (21,22). The code SRIM was used to model the entrance and exit proton energies. End-of-bombardment (EOB) yields were determined using Mathcad14.0 and Equation 1, where N_A is Avogadro's constant (atoms/mol), I is incident particle flux (particles/s), A_t is the atomic weight (g/mol), λ is the decay constant (1/s), t is

irradiation time (s), E_0 is initial proton energy, E_e is exit proton energy, $\sigma(E)$ is the energy-dependent reaction cross section (cm^2) and $S(E)$ is the total stopping power ($\text{MeV}/\text{cm}^2/\text{g}$) (23).

$$Y_{\text{EOB}} = \frac{N_{\text{AI}}}{A_{\text{T}}} (1 - e^{-\lambda t}) \int_{E_e}^{E_0} \sigma(E) \frac{dE}{S(E)} \quad (1)$$

Determination of Equilibrium Dissociation Constant

The equilibrium dissociation constant (K_d) was determined following a previously reported method (24). The K_d values were calculated using Equation 2, where A_i is the initial radioactivity (Bq), A_{eq} is the radioactivity in filtered supernatant (Bq), V is the volume of the filtered supernatant (mL) and m is the mass of the resin (g).

$$K_d = \frac{C_{\text{resin}}}{C_{\text{aqueous}}} = \frac{A_i - A_{\text{eq}}}{A_{\text{eq}}} \cdot \frac{m}{V} \quad (2)$$

Digestion of Ti Foil and TiO_2

Irradiated Ti was transferred into a conical 15mL screw-cap perfluoroalkoxy alkane (PFA) vial (Savillex, Eden Prairie, MN) containing approximately 200mg of NH_4HF_2 dissolved in 3mL of 12.1M HCl. The vial was capped and heated at 230°C for 45min. Irradiated TiO_2 was transferred into a conical 15mL screw-cap vial and mixed with NH_4HF_2 (1:3 ratio). The vial was capped and heated to 230°C for 45min. After heating, the dry residue was dissolved by adding 5mL of 12.1M HCl and heating the closed vessel for 45min in a Si-oil bath at 160°C. For both digestion methods, samples were taken in triplicate, filtered, and diluted in 2% HNO_3 for trace metal analyses. The digestion methods are illustrated in Figure 2.

BDGA Separation

Approximately 110mg of BDGA resin was vortexed with 5mL of 7M HNO₃. The mixture was transferred into a 0.8 x 9cm polypropylene column (Bio-Rad, Hercules, CA), the HNO₃ was eluted, and a glass wool plug was placed on the resin bed (7mm height). A flow-rate of 1.0mL/min was used for each eluent. The resin was washed with 5mL each of 7M HNO₃, water, 0.1M HCl and 7M HCl. The digestant was loaded onto the column in approximately 7 to 8M HCl. Eluent 1 (20mL, 7M HCl), eluent 2 (10mL, 7M HNO₃) and eluent 3 (10mL, 0.1M HCl) were passed through the column and collected. The final eluate containing the Sc radionuclides was collected into a 10mL conical vial and evaporated to dryness under vacuum at 100°C. After evaporation, the purified Sc was reconstituted in 0.5M CH₃CO₂NH₄ buffer (pH4.7). The separation method is shown in Figure 3.

Samples were taken from each eluate and analyzed to determine the distribution of Ti and V and the percent recovery of Sc. The decontamination factor, a measure of the Sc purification, was determined using Equation 3, where $D_{A,B}$ is the factor giving the change in ratio of constituent 1 (A) to constituent 2 (B) (25).

$$D_{A,B} = \frac{Q_A}{Q_{A,0}} \times \frac{Q_{B,0}}{Q_B} \quad (3)$$

[^{43,44,47}Sc]Sc-PSMA-617 Radiolabeling and Cell Uptake Studies

The bioconjugate PSMA-617 was labeled with ^{43,44,47}Sc (10MBq/nmol) and characterized by HPLC using two solvents. Solvent A was acetonitrile (ACN) with 0.1% trifluoroacetic acid (TFA) and Solvent B was water with 0.1% TFA. The linear gradient was

5%(A):95%(B) to 80%(A):20%(B) over 15min at 0.7mL/min. The specific uptake of the radioligand to PSMA receptors was demonstrated via a blocking study using 100 μ m of the PSMA inhibitor 2-(phosphonomethyl)pentanedioic acid (PMPA). The PSMA-negative PC3 cell line was used as a control. The concentration of the inhibitor was 100 μ M.

Alkali Precipitation of Ti

Eluate containing Ti was diluted to 500mL with water and heated for 45min at 160°C. While stirring, the pH was adjusted to 8 by dropwise addition of 19mL of 25% ammonia solution. The stir bar was removed and the precipitate was allowed to settle for 1h. The mixture was vacuum filtered with a grade 43 ashless filter paper (GE Healthcare Life Sciences, Pittsburgh, PA) and rinsed twice with 10mL 1% aqueous ammonia solution. The precipitate was washed with 10mL of acetone. The filter paper was transferred to a zirconia crucible and combusted in a tube furnace at 1000°C for 24h.

High Purity Germanium Spectroscopy (HPGe)

Radioactive samples were characterized by gamma-ray spectroscopy using a Canberra GC2018 HPGe detector with an intrinsic efficiency of 24.5% connected to a DSA-100 multichannel analyzer (Meriden, CT). Canberra Genie 2000 software was used for data acquisition and analysis. An energy and efficiency calibration was performed using a mixed nuclide source in a sealed 1.5mL microcentrifuge vial prepared by Eckert & Ziegler Analytics (Atlanta, GA). Efficiency-calibration spectra with a minimum photopeak area of 2×10^5 counts were collected and fit to determine the relative detector efficiency. For all measurements, aliquots of sample material were diluted to 1mL in a 1.5mL vial, and

suspended in an acrylic holder at a distance of 5 or 25mm from the HPGe detector. These geometries were maintained for all sample measurements.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Samples containing stable elements were preserved and analyzed via ICP-MS. Multi-element standards in 2% HNO₃ at 0.1, 1, 10, 100 and 1000µg/L were used for instrument calibration. Samples were diluted in 2% HNO₃ and measured on an Agilent Technologies 7700 ICP-MS (Santa Clara, CA). Agilent Technologies MassHunter software (v4.3) was used for data acquisition and analyses.

Results

Target Irradiations

The Nb target holder was used to irradiate 250µm thick Ti foils ($\rho=4.506\text{g/cm}^3$) at 24MeV and tolerated beam currents up to 40µA. The average ($n=3\pm 1\sigma$) mass of a Ti foil was (91.5±0.3)mg. A two-piece Al target holder was used to irradiate a 400µm thick pressed TiO₂ ($\rho=4.23\text{g/cm}^3$) disc at 17MeV. The average ($n=3\pm 1\sigma$) mass of the disc target was (52.2±0.4)mg. The magnetization of the SmCo magnets was confirmed after each irradiation ($n=6$). At a beam current of 20µA, thermal damage was observed on the Al cap and base; thus, subsequent irradiations were limited to 15µA. The Ta target holder was used to irradiate a 400µm thick pressed TiO₂ disc at 24MeV at beam currents up to 40µA for 30min with no damage to the target holder or material. The average ($n=3\pm 1\sigma$) mass of the disc target was (110.1±0.5)mg.

Digestion Methods

Typical digestion times for Ti foils and pressed TiO₂ discs were 45 and 90min, respectively. Titanium foils were digested at 160°C using a mixture of NH₄HF₂ and 12.1M HCl. Trace metals analysis of the digestant via ICP-MS showed that the average ($n=3\pm 1\sigma$) percentage of Ti foil dissolved in solution was (98±3)%. TiO₂ discs were digested in two-steps and the average ($n=3\pm 1\sigma$) percentage of TiO₂ dissolved in solution was (95±1)%. These methods were developed using non-irradiated Ti target material and data from initial experiments are shown in Supplemental 1.

Scandium-43, ^{44g,44m}Sc, ⁴⁷Sc, and ⁴⁸V Yields From ^{nat}Ti Targets

The radionuclides measured by HPGe were ⁴⁸V ($t_{1/2}=15.97\text{d}$, $\gamma_1=944.1$, $\gamma_2=983.5$, $\gamma_3=1312.1\text{keV}$), ⁴³Sc (372.9keV), ^{44g}Sc (1157.0keV), ^{44m}Sc (271.2keV) and ⁴⁷Sc (159.4keV). The sample counting time was varied to achieve a minimum photopeak area of 500 counts and dead time was kept below 5%. The average EOB production rate ($n=9\pm 1\sigma$) and predicted rate for each radionuclide are shown in Table 1. These experimental rates were normalized to 1μA.

BDGA Separation

The digestant containing Ti ions, Sc radionuclides, and ⁴⁸V was loaded onto BDGA resin. The bulk Ti target material was eluted from the resin and resulted in an average ($n=6\pm 1\sigma$) Ti recovery factor of (96±5)% in 12mL of the load eluate and 20mL of eluate 1. In contrast, Sc radionuclides were strongly adsorbed onto the resin in 7 to 8M HCl. No Sc radionuclides were detected in the eluates from the load, eluent 1 and eluent 2. The Sc radionuclides were desorbed from the resin in 10mL of eluent 3 (0.1M HCl). The isolated

Sc radionuclides (eluate 3) were free of radionuclidic impurities, including ^{48}V . These data are summarized in Table 2 and they show an average ($n=3\pm 1\sigma$) Sc recovery from Ti foil and TiO_2 irradiated targets of $(94\pm 1)\%$ and $(94\pm 3)\%$, respectively.

Samples taken from the final Sc product after reconstitution showed the presence of non-radioactive contaminants. The identity and average ($n=3\pm 1\sigma$) concentration of contaminants in 400 μL of 0.5M $\text{CH}_3\text{CO}_2\text{NH}_4$ were Fe $(1.0\pm 0.3)\text{mg/L}$, Cu $(0.8\pm 0.3)\text{mg/L}$, Zn $(1.3\pm 0.3)\text{mg/L}$, V $(0.5\pm 0.3)\text{mg/L}$, and Al $(0.8\pm 0.3)\text{mg/L}$. Scandium was not detected or was below the detection limits of the ICP-MS used in this work. The complete separation and concentration of Sc radionuclides required approximately 180min.

$^{43,44,47}\text{Sc}$]Sc-PSMA-617 Radiolabeling and Specific Uptake in LNCaP Tumor Cells

PSMA-617 was labeled with $^{43,44,47}\text{Sc}$ in 0.5M $\text{NH}_4\text{CH}_3\text{CO}_2$ at 95°C for 30min. A $>99\%$ radiochemical purity (10MBq/nmol) was measured by HPLC (Figure 4). The average ($n=4\pm 1\sigma$) percent of $^{43,44,47}\text{Sc}$]Sc-PSMA-617 associated with LNCaP cells (Supplemental 2) was $(16.6\pm 1.5)\%$ after 2h and $(21.9\pm 1.7)\%$ after 4h of incubation at 37°C . The average ($n=4\pm 1\sigma$) non-specific uptake in PC3 cells (control) and in the presence of PMPA was $<0.5\%$.

Ti Recycling

The combined eluates containing Ti were diluted and pH adjusted. Near pH8 (verified by pH strip test), a fine white cloudy precipitate formed. After filtration and combustion, the average ($n=4\pm 1\sigma$) percent recoveries of Ti were $(103\pm 5)\%$ and $(108\pm 8)\%$ from Ti foil and TiO_2 , respectively.

Discussion

Targets were created for the irradiation of TiO₂ at beam current up to 40μA and energy up to 24MeV. The SmCo magnets (Al target) did not lose their magnetism and could play a role in the design of compact cyclotron targets. The Ta target was robust and future work will be carried out to define the maximum tolerated beam current on TiO₂. Further, these data show that ^{43,44,47}Sc can be produced via TiO₂ at beam currents greater than those currently used for cyclotron production via CaO or CaCO₃ (approximately 20μA).

The EOB production rate of Sc radionuclides and co-produced ⁴⁸V at 24MeV in Ti foils agreed with the predicted rate based on published nuclear data sets for the ^{nat}Ti(p,x) reaction (Table 1). Using the same methodology, the EOB yields of ^{43,44,47}Sc from enriched TiO₂ target material were predicted for the ^{46,47,50}Ti(p,α) nuclear reactions (Supplemental 3) at 1, 4, and 24h (7,26,27). These yields (MBqs-GBqs) obtained from enriched Ti nuclei would be suitable for pre-clinical and in some cases clinical investigations.

Previously reported methods for digestion of TiO₂ utilized concentrated HF with some methods requiring hours to reach completion (2-6). These methods could result in the loss of at least one ^{43,44}Sc half-life. The digestion methods in this work use pressure, heat, and the in situ generation of approximately 1.0M HF via the reaction of NH₄HF₂ with 12.1M HCl (28). The results showed that greater than 95% of Ti foil and TiO₂ was dissolved in 45 and 90min, respectively. The closed PFA vessels used in this work were inexpensive and durable at temperatures up to 250°C. These data support future efforts to characterize and optimize the digestion parameters to further decrease digestion times for Ti foil and TiO₂.

Branched DGA and DGA resins have been used to purify Sc in multiple reports (8,17,29). Based on equilibrium dissociation constants determined in this work for Sc, Ti and V (Supplemental 4), the separation method developed in this work was predicted to separate Sc from Ti and V, and was confirmed by determining the Sc decontamination factor relative to each contaminant. Based on Table 2, the decontamination factor for ^{47}Sc relative to ^{48}V was greater than 6×10^6 and 3×10^6 for Sc isolated from Ti foil and TiO_2 , respectively. Similarly, Ti present in the recovered Sc fractions was below the ICP-MS detection limits (approximately $1 \mu\text{g/L}$); thus, the data shows the decontamination factor of ^{47}Sc relative to Ti was greater than 2.8×10^5 and 1.6×10^5 for Ti foil and TiO_2 , respectively. These data show that Sc was isolated with little contamination from Ti and V. The overall Sc recovery factors agreed with previously reported procedures using DGA resins (8,17,30). Trace metal analyses via ICP-MS of the Ti containing digestants and recovered Sc fractions showed the presence of Fe, Cu, Zn, V and Al. These transition metal contaminants can reduce radiolabeling yield by competing with Sc radionuclides for ligand binding. The sources of these impurities can be from the target material or environmental contamination. The average concentrations of these impurities were between 0.5-1.3mg/L, which compared well to previous reports (17,30).

To demonstrate the efficacy of these methods, a proof-of-concept study was conducted using PSMA-617 radiolabeled with Sc. The results of the study showed specific-targeting of the PSMA receptors on LNCaP cells using $^{[43,44,47}\text{Sc}]\text{Sc-PSMA-617}$ at a molar activity of 10MBq/nmol.

Historically and in recent reports, Ti has been precipitated with aqueous ammonia solutions at pH8 (8,31). In this work, a Ti precipitant was obtained using an aqueous

ammonia solution to adjust the bulk media to pH8. After sufficient time for aggregation and sedimentation, the precipitate was filtered and combusted. The typical concentration of Ti in the filtrate was below 100µg/L (approximately 50µg) which indicated the percent loss of Ti after precipitation was less than 0.1%. The mass recoveries of Ti exceeded 100%, which may be attributed to two factors: (1) etching of glass wool by HF generated in situ and (2) the incomplete combustion of the filter paper or presence of water. These data show that the recovery of Ti in the oxide form with aqueous ammonia solution is an effective approach to recycling Ti. Additional work will be performed to reduce the introduction of impurities to avoid buildup of contaminants during iterative uses of this method.

Conclusion

In this study we investigated the feasibility of producing the theranostic radionuclides of Sc on a proton accelerator using a robust and unified process. Results from this work showed that routine production of $^{43,44,47}\text{Sc}$ could be accomplished using enriched TiO_2 . Importantly, our findings showed that TiO_2 targets may tolerate beam currents greater than 30µA using the target holders designed in this work, TiO_2 can be digested via a two-step process in 90min, and digested Ti may be efficiently recovered via alkali-precipitation with aqueous ammonia solution. Future work should investigate the maximum tolerated beam current on TiO_2 using these target holders and any impact on downstream chemistry which may be caused by the increased heat deposition in the target material. Additionally, these efforts should seek to better understand and optimize the digestion methods developed in this work in order to further reduce digestion times. Finally, the method to precipitate Ti should be modified to remove sources of silicon. Ultimately, the

results in this work showed the potential promise in using enriched Ti targets to produce these matched-pair theranostic radionuclides of Sc.

Question

Is production of the theranostic radionuclides of Sc feasible using enriched TiO₂ as a target material?

Pertinent Findings

The theranostic radionuclides of Sc were produced using naturally isotopic TiO₂ on a proton accelerator at energies up to 24MeV. These targets were rapidly digested in 90min followed by Sc purification using ion-exchange chromatography. This work showed a 94% recovery of Sc radionuclides and demonstrated a simple method to recover digested target material.

Implications for Patient Care

The findings in this work add to efforts aimed at identifying easily adoptable methods to produce theranostic radionuclides of Sc which are increasingly being used in clinical and pre-clinical studies.

Conflict of Interest Statement

The author(s) declare(s) that there is no conflict of interest.

References

1. Kinsey RR, Dunford CL, Tuli JK, Burrows TW. *The NUDAT/PCNUDAT Program for Nuclear Data*. Data extracted from the NUDAT database, v2.8, February 17th, 2020.
2. Maurer T, Eiber M, Schwaiger M, Gschwend JE. Current use of PSMA-PET in prostate cancer management. *Nat Rev Urol*. 2016;13:226-235.
3. Hennrich U, Kopka K. Lutathera®: The First FDA- and EMA-Approved Radiopharmaceutical for Peptide Receptor Radionuclide Therapy. *Pharmaceuticals*. 2019;12.
4. Wester HJ, Schottelius M. PSMA-Targeted Radiopharmaceuticals for Imaging and Therapy. *Semin Nucl Med*. 2019;49:302-312.
5. Müller C, Domnanich KA, Umbricht CA, van der Meulen NP. Scandium and terbium radionuclides for radiotheranostics: current state of development towards clinical application. *Br J Radiol*. 2018;91:20180074.
6. Eppard E, de la Fuente A, Benešová M, et al. Clinical Translation and First In-Human Use of [⁴⁴Sc]Sc-PSMA-617 for PET Imaging of Metastasized Castrate-Resistant Prostate Cancer. *Theranostics*. 2017;7:4359-4369.
7. Carzaniga TS, Auger M, Braccini S, et al. Measurement of ⁴³Sc and ⁴⁴Sc production cross-section with an 18MeV medical PET cyclotron. *Appl Radiat Isotopes*. 2017;129:96-102.
8. Domnanich KA, Eichler R, Müller C, et al. Production and separation of ⁴³Sc for radiopharmaceutical purposes. *EJNMMI Radiopharm Chem*. 2017;2:14-14.
9. Walczak R, Krajewski S, Szkliniarz K, et al. Cyclotron production of ⁴³Sc for PET imaging. *EJNMMI Phys*. 2015;2:33.
10. Krajewski S, Cydzik I, Abbas K, et al. Cyclotron production of ⁴⁴Sc for clinical application. *Radiochim Acta*. 2013;101:333-338.
11. Duchemin C, Guertin A, Haddad F, Michel N, Métivier V. Production of scandium-44m and scandium-44g with deuterons on calcium-44: cross section measurements and production yield calculations. *Phys Med Biol*. 2015;60:6847-6864.
12. Severin GW, Engle JW, Valdovinos HF, Barnhart TE, Nickles RJ. Cyclotron produced ^{44g}Sc from natural calcium. *Appl Radiat Isot*. 2012;70:1526-1530.
13. Szkliniarz K, Sitarz M, Walczak R, et al. Production of medical Sc radioisotopes with an alpha particle beam. *Appl Radiat Isot*. 2016;118:182-189.
14. Frank R. Scandium-44: Benefits of a Long-Lived PET Radionuclide Available from the ⁴⁴Ti/⁴⁴Sc Generator System. *Curr Rad*. 2012;5:187-201.

15. Domnanich KA, Müller C, Benešová M, et al. ^{47}Sc as useful β -emitter for the radiotheragnostic paradigm: a comparative study of feasible production routes. *EJNMMI Radiopharmacy and Chemistry*. 2017;2:5.
16. Loveless CS, Radford LL, Ferran SJ, Queern SL, Shepherd MR, Lapi SE. Photonuclear production, chemistry, and in vitro evaluation of the theranostic radionuclide ^{47}Sc . *EJNMMI Research*. 2019;9:42.
17. Rotsch DA, Brown MA, Nolen JA, et al. Electron linear accelerator production and purification of scandium-47 from titanium dioxide targets. *Appl Radiat Isot*. 2018;131:77-82.
18. Mamtimin M, Harmon F, Starovoitova VN. Sc-47 production from titanium targets using electron linacs. *Appl Radiat Isot*. 2015;102:1-4.
19. Carzaniga TS, Braccini S. Cross-section measurement of $^{44\text{m}}\text{Sc}$, ^{47}Sc , ^{48}Sc and ^{47}Ca for an optimized ^{47}Sc production with an 18 MeV medical PET cyclotron. *Appl Radiat Isot*. 2019;143:18-23.
20. Ziegler JF, Ziegler MD, Biersack JP. SRIM – The stopping and range of ions in matter. *Nucl Instrum Methods Phys Res B*. 2010;268:1818-1823.
21. Zerkin VV, Pritychenko B. The experimental nuclear reaction data (EXFOR). *Nucl Instrum Methods Phys Res A*. 2018;888:31-43.
22. Khandaker MU, Kim K, Lee MW, et al. Investigations of the $^{nat}\text{Ti}(p,x)^{43,44\text{m},44\text{g},46,47,48}\text{Sc},^{48}\text{V}$ nuclear processes up to 40MeV. *Appl Radiat Isot*. 2009;67:1348-1354.
23. Otuka N, Takács S. Definitions of radioisotope thick target yields. *Radiochimica Acta*. Vol 103; 2015:1.
24. Radchenko V, Engle JW, Medvedev DG, et al. Proton-induced production and radiochemical isolation of ^{44}Ti from scandium metal targets for $^{44}\text{Ti}/^{44}\text{Sc}$ generator development. *Nucl Med Biol*. 2017;50:25-32.
25. Sandell EB. Meaning of the term "separation factor". *Anal Chem*. 1968;40:834-835.
26. Levkovski VN. *Middle mass nuclides activation cross-sections by medium energy protons and a particles*. Moscow: Inter-Vesi; 1991.
27. Gadioli E, Gadioli Erba E, Hogan JJ, Burns KI. Emission of alpha particles in the interaction of 10–85 MeV protons with $^{48,50}\text{Ti}$. *Zeitschrift für Physik A Atoms and Nuclei*. 1981;301:289-300.
28. Zhang W, Hu Z, Liu Y, Chen H, Gao S, Gaschnig RM. Total Rock Dissolution Using Ammonium Bifluoride (NH_4HF_2) in Screw-Top Teflon Vials: A New Development in Open-Vessel Digestion. *Anal Chem*. 2012;84:10686-10693.

- 29.** Valdovinos HF, Hernandez R, Barnhart TE, Graves S, Cai W, Nickles RJ. Separation of cyclotron-produced ^{44}Sc from a natural calcium target using a dipentyl pentylphosphonate functionalized extraction resin. *Appl Radiat Isot.* 2015;95:23-29.
- 30.** Alliot C, Kerdjoudj R, Michel N, Haddad F, Huclier-Markai S. Cyclotron production of high purity $^{44\text{m}},^{44}\text{Sc}$ with deuterons from $^{44}\text{CaCO}_3$ targets. *Nucl Med Biol.* 2015;42:524-529.
- 31.** Gooch FA. On the Separation of Titanium and Aluminum, with a Note on the Separation of Titanium and Iron. *Proc Am Acad Arts Sci.* 1884;20:435-447.

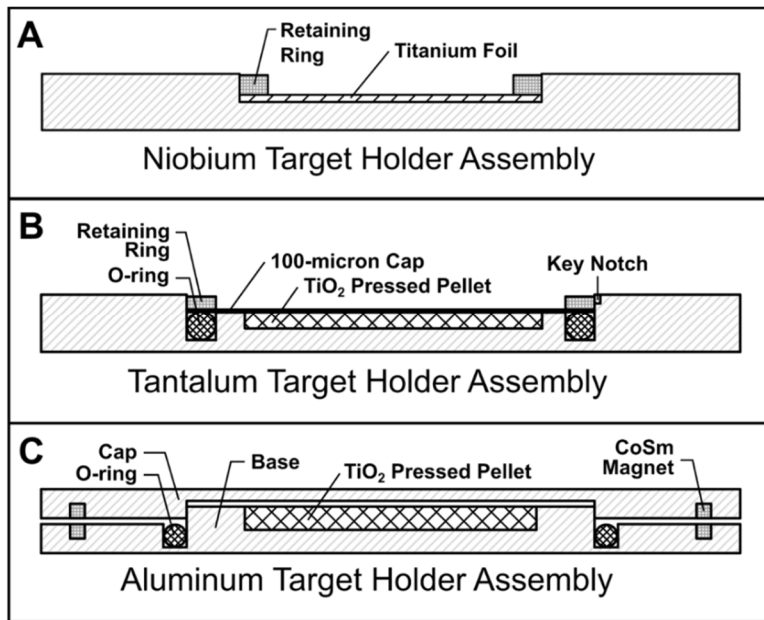


Figure 1. Target holder assemblies tested in this work.

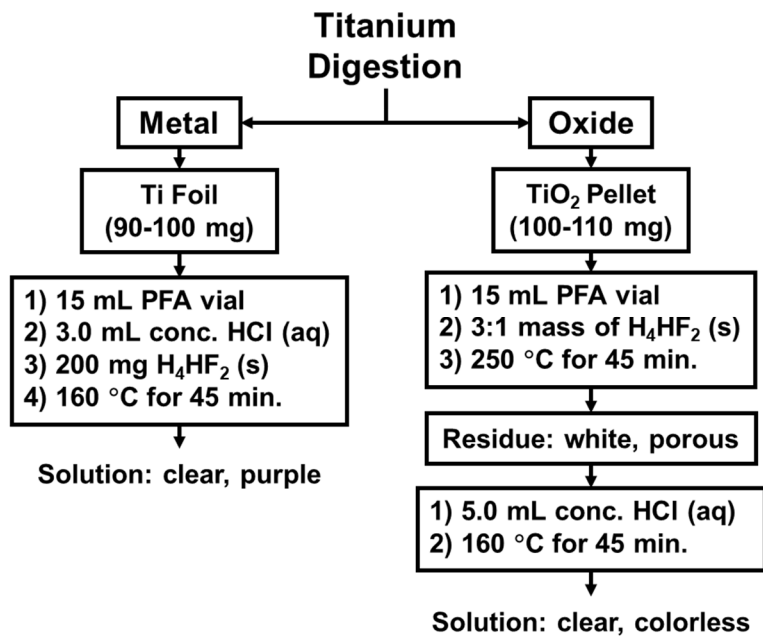


Figure 2. Methods for the digestion of Ti foil and TiO₂.

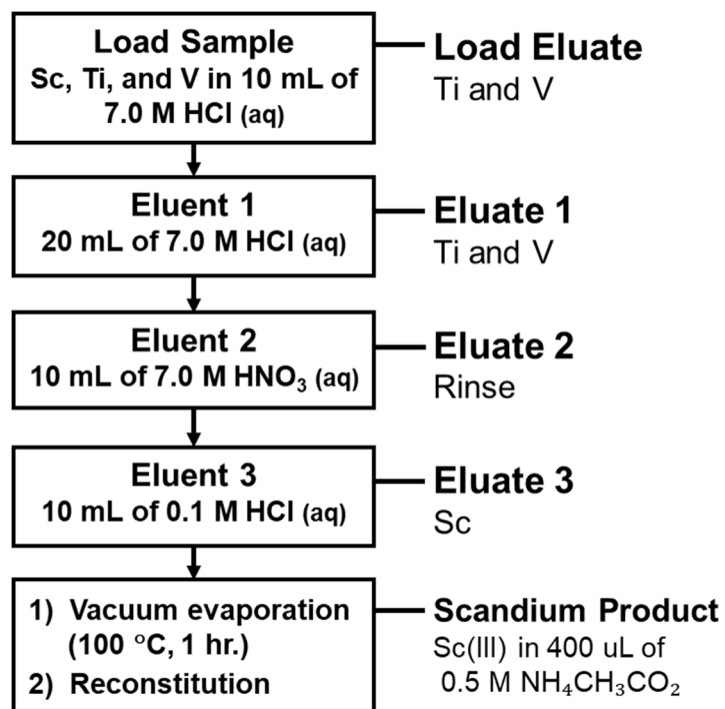


Figure 3. Schematic of the ^{43,44,47}Sc separation.

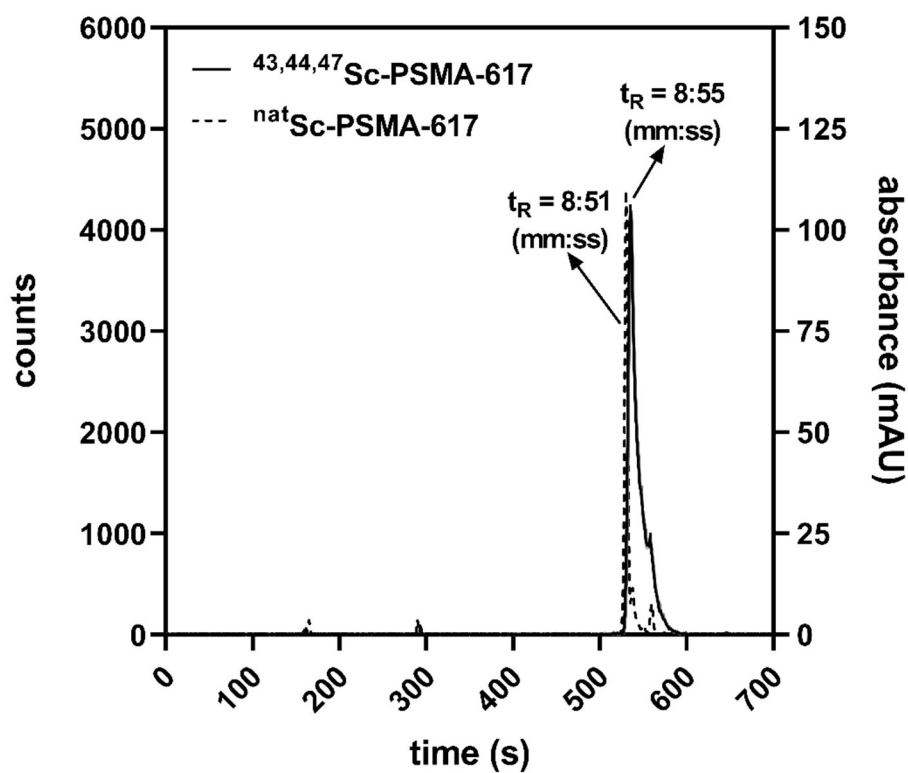


Figure 4. The solid trace (L) show a radiochromatogram of $^{[43,44,47}\text{Sc}]\text{Sc-PSMA-617}$. The dashed trace (R) shows the absorbance of $^{\text{nat}}\text{Sc-PSMA-617}$ at 280nm.

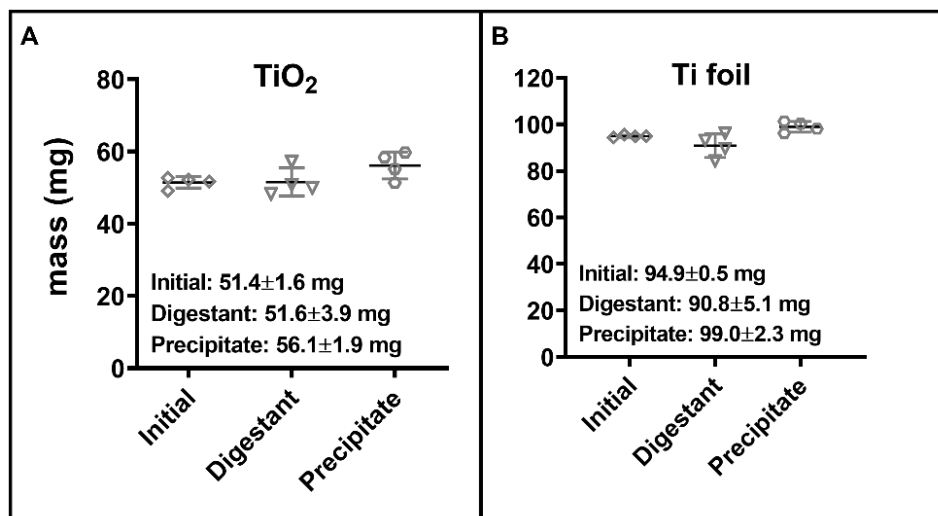
Table 1. The average ($n=9\pm 1\sigma$) measured and predicted radioactivity of radionuclides, and the average production rate normalized to 1 μA (21,22).

Radionuclide	Predicted rate($\text{kBq}\cdot\text{s}^{-1}\cdot\mu\text{A}^{-1}$)	Avg. rate($\text{kBq}\cdot\text{s}^{-1}\cdot\mu\text{A}^{-1}$)
^{43}Sc	1.1	1.1(0.2)
$^{44\text{g}}\text{Sc}$	4.3	4.2(0.4)
$^{44\text{m}}\text{Sc}$	0.20	0.11(0.01)
^{47}Sc	0.19	0.17(0.01)
^{48}V	0.23	0.25(0.01)

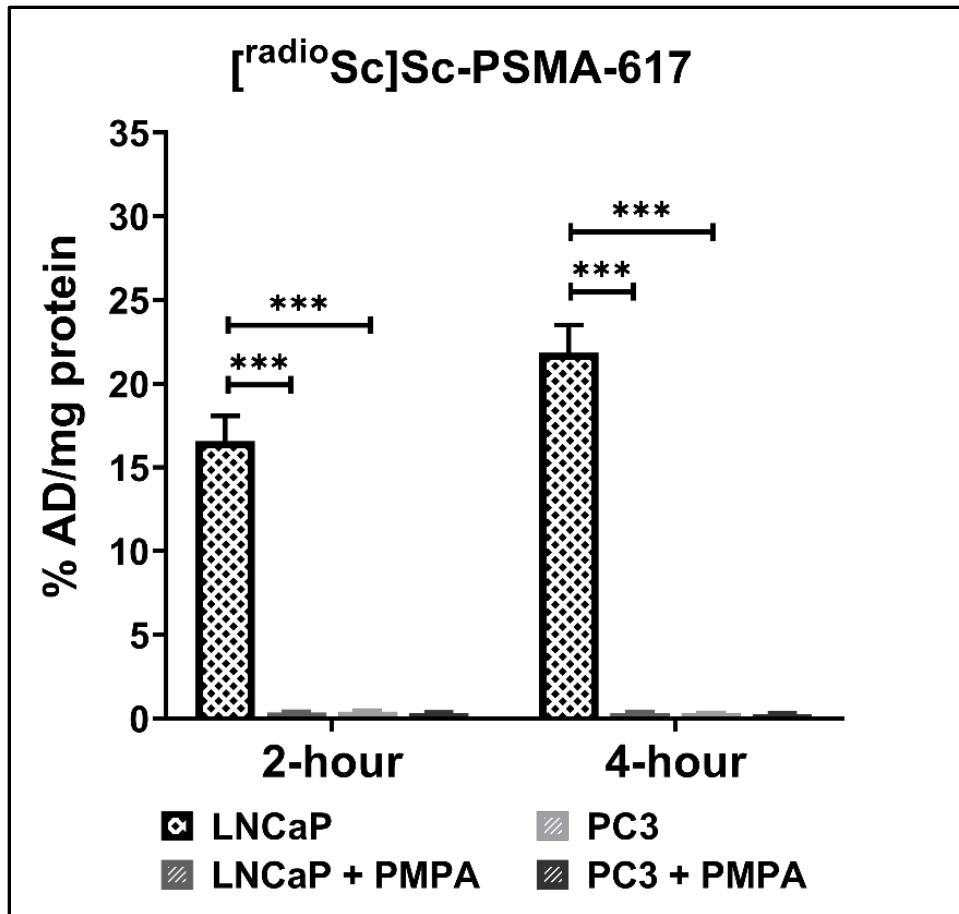
Table 2. The average ($n=3\pm 1\sigma$) initial ^{47}Sc and ^{48}V radioactivity and its recovery in each eluate.

Material	Radionuclide	Initial(MBq)	Load(MBq)	E1(MBq)	E2(MBq)	E3(MBq)
Ti Foil	^{47}Sc	52.3(0.4)	5×10^{-5} *	1×10^{-5} *	1×10^{-5} *	49.0(0.5)
	^{48}V	66.2(0.6)	63.5(0.3)	2.5(0.3)	0.4(0.1)	1×10^{-5} *
TiO ₂	^{47}Sc	29.1(0.7)	3×10^{-5} *	1×10^{-5} *	1×10^{-5} *	27.6(0.6)
	^{48}V	38.4(0.8)	35.1(0.2)	3.1(0.3)	0.3(0.1)	1×10^{-5} *

*Minimum Detectable Radioactivity



Supplemental 1. Pressed Non-radioactive TiO₂ disc targets (A) and Ti foils (B) were prepared in quadruplicate ($n=4\pm 1\sigma$). The samples were weighed, dissolved, and Ti ions in the digestant were precipitated using methods detailed in this work. The percent of TiO₂ dissolved and Ti recovered via precipitation were $(100\pm 8)\%$ and $(108\pm 11)\%$, respectively. Similarly, the percent of Ti foil dissolved and Ti recovered via precipitation were $(96\pm 5)\%$ and $(108\pm 7)\%$, respectively.



Supplemental 2. LNCaP (PSMA+) and PC3 (PSMA-) cells were seeded in 24-well plates in 0.5mL/well of RPMI+ (RPMI-1640 containing 10% FBS) and incubated for 48h at 37°C in 5% CO₂ at a concentration of 1x10⁵ cells/well and 0.5x10⁵ cells/well, respectively. The supernatant was removed and the cells were washed once with warm PBS before addition of 0.5mL of RPMI+ containing 2.0nM of each radioligand (10MBq/nmol) or 0.5mL of RPMI+ containing a mixture of 2.0nM of each radioligand and 100µM of 2-PMPA. The sample size for each blocking and non-blocking cell line was six. The cells were incubated for 2 and 4h at 37°C in a 5% CO₂ atmosphere, washed twice with 0.5mL of ice-cold PBS, and lysed with 200uL 0.2N. The lysate was transferred into a 1.5mL centrifuge tube. Each tube was measured in a gamma counter. The results were expressed as percentage of

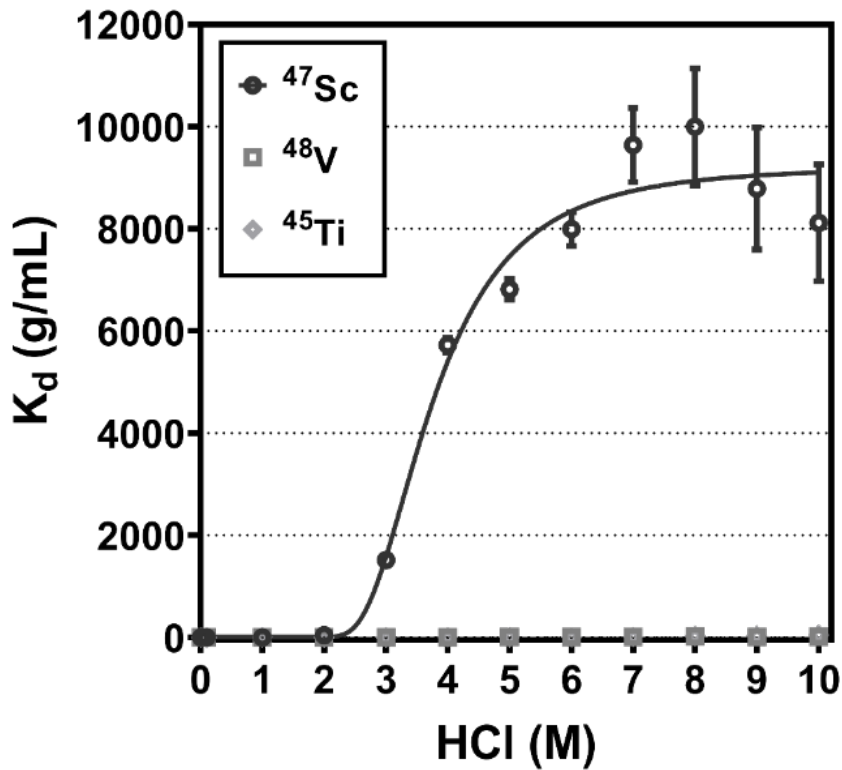
the ratio of cell-associated activity to activity added to each well and graphs were prepared using GraphPad Prism software (v.7.0).

Supplemental 3. The predicted yield of Sc radioisotopes based on the irradiation of a 100% enriched 1mm thick TiO₂ target at 1, 4 and 24h. The yields were based on an entry and exit proton energy of 24 and 15.5MeV at 20μA and published cross section data (6,17,22,23). The nuclear reactions modeled were ⁴⁶Ti(p,α)⁴³Sc, ⁴⁷Ti(p,α)^{44g}Sc, ⁴⁷Ti(p,α)^{44m}Sc, ⁵⁰Ti(p,α)⁴⁷Sc and ⁴⁸Ti(p,2p)⁴⁷Sc.

Time	⁴³ Sc(GBq)	^{44g} Sc(GBq)	^{44m} Sc(MBq)	⁴⁷ Sc(MBq)
1h	2.1	2.6	70	60* 30 [†]
4h	6.5	8.2	270	230* 130 [†]
24h	12.6	16.1	1500	1200* 700 [†]

*Predicted yield from ⁵⁰Ti(p,α)⁴⁷Sc

[†]Predicted yield from ⁴⁸Ti(p,2p)⁴⁷Sc



Supplemental 4. A mixture of 100mg BDGA resin, 990 μ L of HCl (0.1 to 10M) and 10 μ L of ^{48}V (100kBq/mL), ^{47}Sc (500kBq/mL) or ^{45}Ti (222MBq/mL) were prepared in quadruplicate to determine the average ($n=4\pm 1\sigma$) K_d . Each sample was equilibrated on a rocker for 24h after which the supernatant was removed and filtered with a 0.22 μ m hydrophilic membrane filter. A 100 μ L aliquot of the filtered supernatant was diluted to 1mL with water for each sample and measured with an HPGe detector. Likewise, a 100 μ L aliquot of the filtered supernatant was diluted to 10mL with 2% HNO_3 for each sample and measured via ICP-MS. At an HCl concentration of 6 to 10M, no photopeak was observed for ^{47}Sc . The minimum detectable radioactivity (MDA) for ^{47}Sc was calculated based on a 2h measurement of the supernatant. These MDA quantities were used to calculate the K_d for these samples.