Radiochemical isolation of ^{45}Ti using ion chromatography

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Abstract

^{45}Ti exhibits favorable decay properties for positron emission tomography (PET) imaging and can be easily produced by the bombardment of natural scandium (Sc) by protons using the ^{45}Sc(p,n)^{45}Ti nuclear reaction. However, separation of ^{45}Ti from irradiated Sc targets is arduous due to the hydrolytic instability of Ti(IV) complexes, making it a significant bottleneck for routine application of this radionuclide. In the present work, we describe the development and optimization of a ion chromatographic separation method based on trapping of ^{45}Ti on a hydroxamate-functionalized chelating resin and subsequent elution with oxalic acid at pH = 2.8. Under optimized conditions, this method enabled ^{45}Ti-recovery of 61±6% within 8 min. Sc contamination in scaled-up experiments was found to be only 3±1.8µg/mL . The resulting ^{45}Ti-solution was directly used for complexation with CDTA as a model chelator affording the corresponding [^{45}Ti]Ti(cdta) complex with a radiochemical conversion of 73±3%. Conclusively, this promising method could be transferred to automated synthesis modules and should enable the preparation of ^{45}Ti-labeled compounds for PET imaging.

Keywords

Titanium-45; column chromatography; radiolabeling; radiochemical separation

Introduction

In recent years, there has been growing interest in the use of non-standard radionuclides for advanced medical applications. This emerging trend is partly rooted in increasing demand for long-lived radionuclides for positron emission tomography (PET) imaging of slow (patho)physiological or biodistribution processes. In addition, significant progress in the application of radiometals for endotherapeutic purposes has spurred the need for novel metal-based PET isotopes that can be utilized in the framework of theranostic approaches [1–7] .

Among the available non-standard PET radionuclides, titanium-45 (^{45}Ti) stands out due to its favorable decay properties ($T_{½}$=3.1 h, $I_{\beta+}$= 85%, $E_{\beta+,max}$ = 1.04 MeV). Thus, compared to other radiometals (e.g., gallium-68, scandium-43/44 or copper-61/64 [8–11]), ^{45}Ti exhibits a low β^+-energy and negligible γ-radiation [11] , which results in superior PET images and makes it a prime candidate for labeling of peptides and other biomolecules [12]. However, the fast hydrolysis of Ti(IV) complexes remains a significant impediment to the routine utilization of this radionuclide, since it hampers isolation of ^{45}Ti from the target material and complicates the synthesis of stable radiocomplexes.

Efficient separation of ^{45}Ti from irradiated Sc has been addressed by several working groups in the past. To To assess the effectiveness of the method, different factors have to be considered like duration of separation, the purity of ^{45}Ti, and the simplicity of handling the high levels of radioactivity involved. Table 1 provides a comprehensive overview of various separation techniques as described in the literature. The highest ^{45}Ti recovery and lowest amount of Sc impurities was reported for liquid-liquid extraction by Pedersen et al. [13]. In this method a solvent mixture of guaiacol/anisole was applied to extract ^{45}Ti from a hydrochloric acid solution using a dedicated in-flow liquid-liquid extraction system. That methods relies on the utilization of specialized membrane filters and the application of solvents with a high-boiling point, which limits the practical applicability of this approach for automated tracer syntheses.

Table 1:Comparison of natSc/45Ti- separation methods.

	Method	**m(target) [mg]**	**% Recovery**	**Sc contamination**	**Separation time [min]**
Pedersen et al. [13]	Liquid-Liquid-Extraction	20-60	90.3±1.1	pg range	Not specified
Giesen et al. [14]	Thermo-chromatography	350±100	76±5	5 µg	115
Chen et al. [15]	Ion chromatography	96-140	42±6	Not specified	60
Severin et al. [16]	Ion chromatography	20-60	93±3	1.4 pg/MBq	Not specified
Gagnon et al. [17]	Ion chromatography	100-120	56±6	Not specified	Not specified
Chaple et al. [18]	Ion chromatography	~60	78±8	0.03 µg	Not specifified
Vavere et al. [19]	Ion chromatography	35	92.3	Not specified	Not specified
Koller et al. [20]	Ion chromatography	10	81.7±5	ppb range	75

More recently, the thermochromatographic separation of ^{45}Ti from Sc targets was investigated in more detail [14, 18] . Thermochromatography enables isolation of the radionuclide in the form of well characterized no-carrier-added (n.c.a.) [^{45}Ti]$TiCl_4$ [14] . However, the air sensitivity of [^{45}Ti]$TiCl_4$, the time-consuming separation process as well as the rather arduous setup have prevented broad implementation of this procedure for routine tracer production.

In addition, several methods based on ion exchange chromatography have been reported in the literature [15–23] , but their practical application is hampered by long separation times, poor availability of the necessary stationary phases, a need for large

amounts of solvents and/or the formation of non-reactive ^{45}Ti species that require additional processing before the radiolabeling step.

The aim of the present work was to establish a rapid chromatographic separation method which is less challenging and provides n.c.a. ^{45}Ti in a chemical form enabling direct subsequent radiolabeling. Encouraged by the results of Radchenko et al. [24] on the production of a ^{44}Sc/^{44}Ti-generator with hydroxamate-functionalized ZR Resin™, we investigated the use of this resin to isolate ^{45}Ti from bulk scandium targets. Originally ZR Resin™ was developed for ^{89}Zr/Y separations but it also shows high selectivity for titanium over scandium [25] . Thus, according to Radchenko et al. [24] , the distribution coefficients (Kd) of Sc and Ti on this resin in hydrochloric acid (0.1 M - 10 M) amounted to less than 3 and more than 1000, respectively. To this end, a separation method was developed and optimized with regard to ^{45}Ti retention on the resin, washing steps and elution conditions. In addition, the solution with ^{45}Ti after separation was subsequently used for proof-of-principle radiolabeling experiments with CDTA as a model chelator.

Experimental

Radionuclide production

^{45}Ti was produced via the ^{45}Sc(p,n)^{45}Ti nuclear reaction in high yields (337 MBq/µA*h) [11] by irradiation of a natural scandium target (330±30 mg) in a copper target holder [15] with 16.9 MeV protons (2 µA for 30 min) at the Baby Cyclotron BC1710 (INM-5; Forschungszentrum Jülich). To minimize coproduction of ^{44}Sc ($T_{1/2}$: 3.9 h) [11] , a 250 µm Cu foil was used to degrade the proton energy to approximately 12 MeV. During the optimization studies, some irradiations were performed without the degrader foil to enable the formation of ^{44}Sc via he (p,pn)-process to monitor the separation and determination of radiochemical purity of Ti from Sc.

Ion chromatographic separation

Based on the work of Radchenko et al. [9] , commercially available ZR Resin™ (Triskem, France) was selected as the stationary chromatographic phase for the separation experiments. Accordingly, Chromabond^{TM} columns were loaded with different amounts of the resin and preconditioned with 10 M HCl as described in detail in Appendix Section 3.1. The irradiated scandium target was then dissolved in 5 mL 10 M HCl. The resulting solution was diluted to 20 mL using 10 M HCl and divided into 1 mL aliquots, which were loaded onto the preconditioned columns. Unless noted otherwise, each column was washed with 5 mL 10 M HCl and an equal volume of type 1 ultra-pure water (MQ H_2O) before the ^{45}Ti was eluted with 2.5 mL of the respective elution solution (Fig. 1). For scaled-up experiments under optimized conditions, the complete target solution (5 mL) or 1 mL aliquots thereof were loaded onto the preconditioned columns without prior dilution. A detailed description of the experimental procedures is provided in the Appendix Section 3.1 – 3.8.

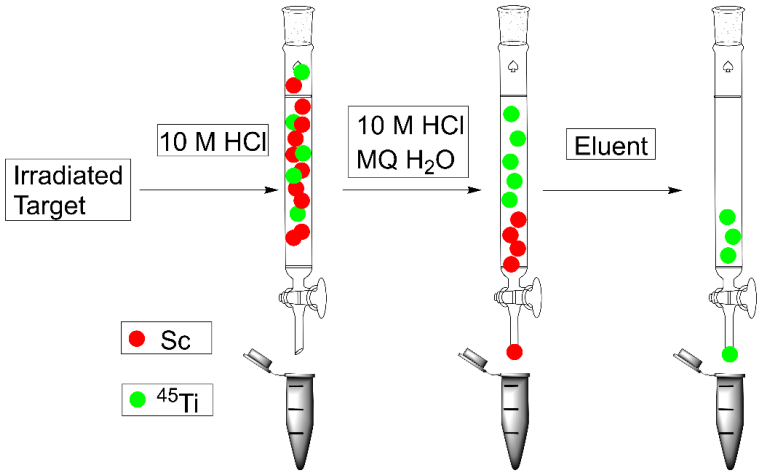


Fig. 1: Scheme of the ^{45}Ti/Sc separation method.

^{45}Ti-Retention

To examine the dependence of ^{45}Ti-retention on the amount of stationary phase, columns were filled with 66±6 mg, 130±6 mg or 280±6 mg of the ZR Resin™ (n=3 per condition). Each column was then loaded with an 1 mL aliquot of the target solution, and the percentage of retained ^{45}Ti was determined.

Recovery of ^{45}Ti in dependence of elution agent

For elution of ^{45}Ti from the ZR Resin™, several weak complexing agents like hydrogen peroxide (H_2O_2), oxalic acid or citric acid in water or mixtures of water and organic solvents were evaluated. (see Appendix 3.3)

Recovery of ^{45}Ti in dependence of pH-value

As the complexation of metal ions can be strongly affected by the pH-value, the elution efficiency of oxalic acid in concentrations ranging from 0.01 to 0.1 M was analyzed at different pH-values (Fig. 4 and Appendix 3.4 Table 2).

Furthermore, the influence of organic solvents on the elution efficiency was evaluated to facilitate transchelation with poorly water-soluble ligands and accelerate removal of the solvent during isolation of the resulting ^{45}Ti-complexes. Therefore, a 0.1 M oxalic acid solution containing 20% MeOH was used.

Radiochemical purity

To assess and minimize the content of Sc in the final product, irradiations were performed without a Cu-degrader foil to produce both ^{45}Ti and ^{44}Sc. The target was then processed with 10 M HCl as described above, aliquots of the resulting target solution were loaded on different columns. Either ^{45}Ti was eluted subsequently with 2.5 mL of 0.1 M oxalic acid or different washing steps were carried out. Washing solutions were 5 mL of water, 10 mL or 15 mL of 10 M HCl followed by an equal amount of water. Finally, the columns were eluted with 0.1 M oxalic acid (pH = 2.8) and the ^{45}Ti: ^{44}Sc ratio in the eluent was determined and compared with the ratio in the original target solution.

Batch experiments

To further determine the amount of Sc-contamination, scaled-up separation experiments under optimized conditions (130 mg ZR Resin™, 5 mL volume of wash solutions, elution with 0.1 M oxalic acid at pH = 2.8) were performed with non-radioactive Sc (350 mg) dissolved in 5 mL 10 M HCl. The solutions obtained by elution of the columns were analyzed by ICP-MS.

Additional experiments under optimized conditions were performed with 1 mL aliquots of the target solution obtained by dissolution of an irradiated Sc target in 5 mL 10 M HCL (6-35 MBq per aliquot).

Finally, to assess the suitability of the method for application in the routine production of radiopharmaceuticals, scaled-up separation experiments under the optimized conditions were performed with the entire target solution obtained by dissolution of an irradiated Sc target in 5 mL 10 M HCL (Sc: 330±30 mg, ^{45}Ti: 100-180 MBq).

Elution profile

Experiments under the optimized conditions were performed. Therefore, aliquots (1 mL) of the target solution (21-29 MBq) were used and the respective eluate was collected in 0.5 mL fractions. The elution profile for Sc was obtained similar by using the non-radioactive Sc solution from the Batch experiment.

Complexation of ^{45}Ti with CDTA

To demonstrate the suitability of the isolated ^{45}Ti for further radiolabeling, a proof-of-principle study was performed with 1,2-cyclohexanedinitrilotetraacetic acid (CDTA) as a model chelator. To this end, CDTA was directly added to the ^{45}Ti solution obtained after elution with either MeCN / 0.65 M H_2O_2 or 0.1 M oxalic acid. Radiochemical conversions (RCCs), defined as the reaction efficiency by measuring the transformation of components in a crude reaction mixture at a given time [26] , were compared with those obtained with n.c.a. [^{45}Ti]$TiCl_4$ separated by thermochromatography [14] . Details on reaction conditions and reference compound are provided in Appendix 4.

Results and discussion

^{45}Ti-Retention

As illustrated in Fig. 2, increasing the amount of ZR Resin™ from 66±6 mg to 130±6 mg improved ^{45}Ti retention from 79.7±5.5% to 92.5±1.7%. Due to the high standard deviation after further increase to 280±6 mg (91.6±16.1%), all subsequent experiments were performed with columns containing 130 mg of the stationary phase.

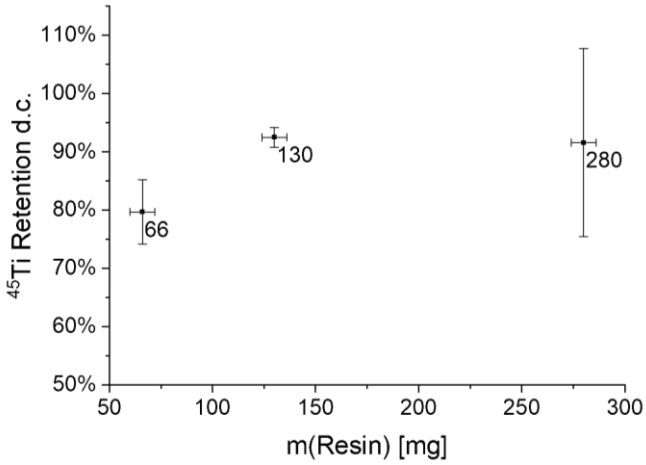
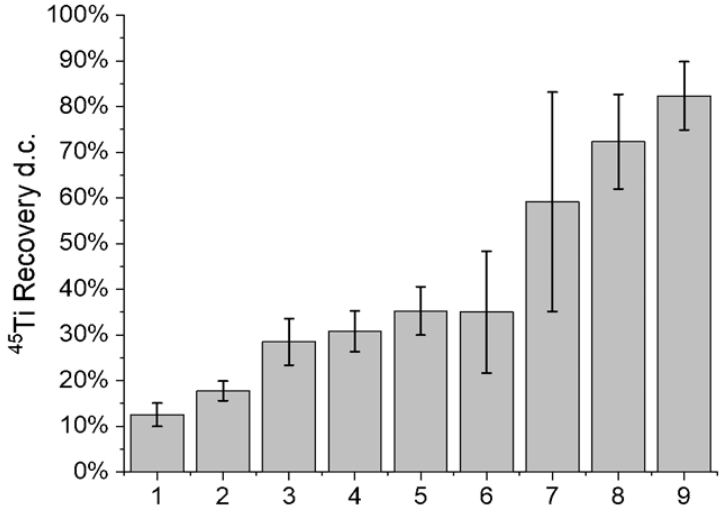


Fig. 2: Decay corrected (d.c.) retention of ^{45}Ti in dependence on the amount of ZR Resin™.

Recovery of ^{45}Ti in dependence of elution agent

Highest recoveries of ~82% were observed when a mixture of acetonitrile (MeCN) and 0.65 M H_2O_2 was used as eluent (Fig. 3). However, this was most likely related to partial elution of the hydroxamate functional groups from the resin due to the high percentage of organic solvent. Elution with pure MeCN resulted also in ~72% recovery. The degradation of the resin was indicated by insoluble components in the eluate.

Among the aqueous elution solutions, 0.5 M oxalic acid showed the best efficiency and eluted around 59% of the ^{45}Ti from the column. Based on this finding and the fact that elution with MeCN-containing solutions proved to hamper subsequent transchelation with other ligands (see Section Labelling of CDTA), oxalic acid was chosen as the eluent of choice for further studies.



No.	Media	Additive c [mol/L]
1	30% MeOH / H_2O / H_2O_2	0.065
2	30% MeCN / H_2O / H_2O_2	0.065
3	Tartaric acid	0.10
4	1 M HCl / H_2O_2	0.065
5	Citric acid	0.10
6	0.65 M H_2O_2	0.65
7	Oxalic acid	0.50
8	MeCN	–
9	MeCN / H_2O_2	0.65

Fig. 3: Decay corrected (d.c.) recovery of ^{45}Ti with different elution solutions.

Recovery of ^{45}Ti in dependence of pH-value

The results showed that the elution efficiency of 0.01 M oxalic acid buffered at either acidic (0.1 M ammonium formate, pH = 3.2) or slightly basic (1.0 M sodium phosphate, pH = 7.9) pH-values was insufficient (<6% recovery). In contrast, elution with unbuffered 0.1 M oxalic acid (pH = 1.3) provided a moderate recovery of 28.9±6.0%, while the elution efficiency decreased when higher pH-values of the buffer solution were applied. However, a higher elution efficiency was observed for 0.05 M oxalic acid solutions, buffered with sodium phosphate at pH-values between 2.6 and 2.8 (36.2±4.6% and 33.4±14.7% recovery). Concentration enhancement of oxalic acid from 0.05 M to 0.1 M increased the recovery of ^{45}Ti almost two-fold (to 65.2±1.2 %).

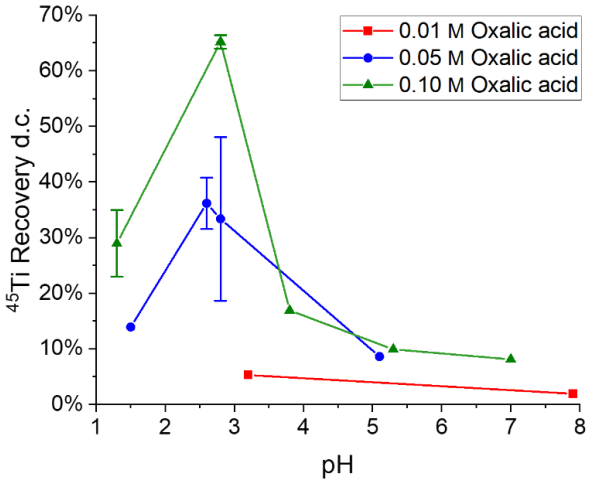
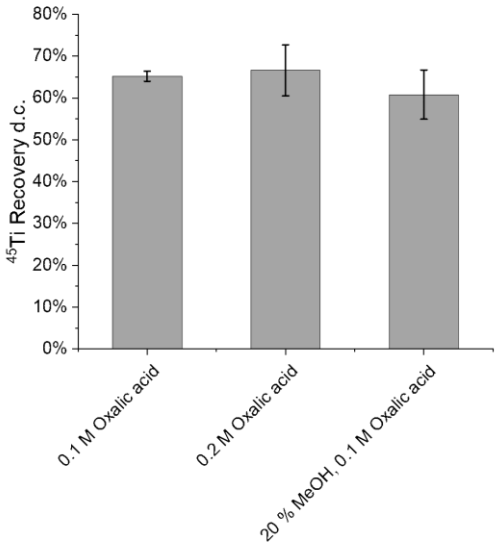


Fig. 4: Decay corrected (d.c.) recovery of ^{45}Ti using oxalic acid solutions in dependence of concentration and pH.

In contrast, doubling the oxalic acid concentration once more to 0.2 M (66.6±6.1) showed no additional effect on ^{45}Ti recovery, as illustrated in Fig. 5.

Fig. 5: ^{45}Ti Recovery with 0.1 M oxalic acid, 0.2 M oxalic acid or 0.1 M oxalic acid in 20% MeOH / phosphate buffer at pH = 2.8.



^{45}Ti recovery with 0.1 M oxalic acid in 20% MeOH / phosphate buffer at pH = 2.8 amounted to roughly 60% and was comparable to the recovery observed without MeOH

(Fig. 5). This suggests that addition of MeOH has no negative effects on the elution efficiency of oxalic acid solutions.

Radiochemical purity

After washing with 5 mL 10 M HCl and 5 mL water resulted in an increase of the $^{45}Ti/^{44}Sc$ ratio to 4000±300. When the volume of the washing solutions was increased from 5 to 10 or 15 mL, the $^{45}Ti/^{44}Sc$-ratio in the eluent showed a progressive decline (Fig. 6), suggesting that higher volumes of washing solutions were contra productive since ^{45}Ti was also co-eluted. As a consequence, 5 mL 10 M HCl and water was considered as the optimal volume for the washing steps in subsequent experiments.

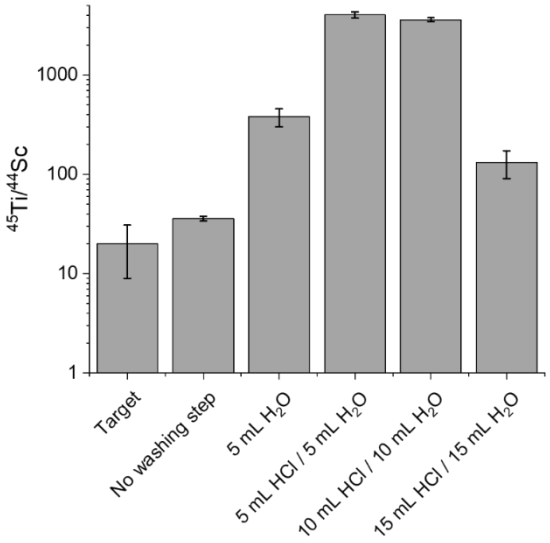


Fig. 6: Comparison of the ^{45}Ti : ^{44}Sc-ratio in the original target solution and the eluents obtained following washing steps with different volumes (5 mL, 10 mL or 1 mL) of 10 M HCl and water.

Batch experiments

The ICP-MS analysis of the eluate indicated an average Sc contamination of 3.0±1.8 µg/mL (for details see Appendix 3.7 A).

Additional experiments under optimized conditions resulted in a decay corrected (d.c.) ^{45}Ti recovery of 69±10% (n=24) (for details see Appendix Section 3.7 B).

The scaled-up separation experiments under the optimized conditions with the entire target solution showed an average separation time of 8 min and the decay corrected ^{45}Ti recovery of 61±8% (n=9) (for details see Appendix Section 3.7 C).

Elution profiles

Fig. 7 shows the elution profiles for ^{45}Ti with 0.1 M oxalic acid at pH = 2.8 (A). With 0.1 M oxalic acid, the largest portion of ^{45}Ti was obtained in the first four 0.5 mL fractions. For comparison, the elution profile for Sc with 0.1 M oxalic acid (B), revealed that the major portion eluted with the second 0.5 mL fraction, is also shown.

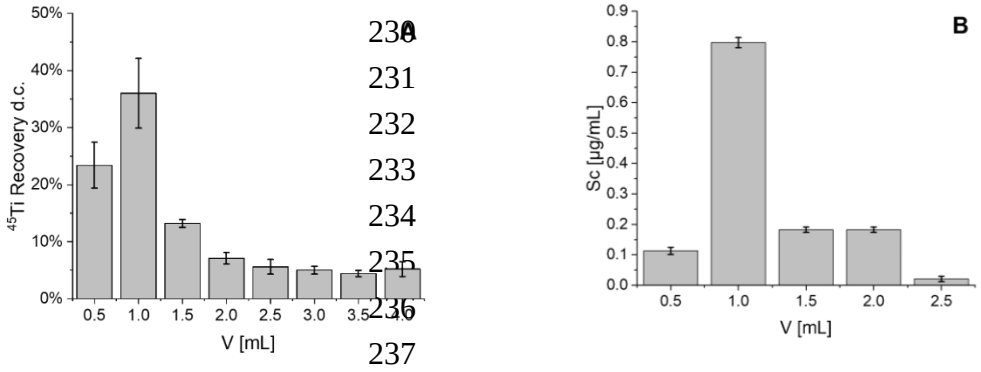


Fig. 7: A: Decay corrected (d.c.) elution profile of ^{45}Ti with 0.1 M oxalic acid at pH = 2.8. B: Elution profile of ^{nat}Sc with 0.1 M oxalic acid at pH = 2.8.

Complexation of ^{45}Ti with CDTA

RCCs of 92±2% obtained with [^{45}Ti]$TiCl_4$ by thermochromatography were slightly higher in comparison to radiolabeling with eluted ^{45}Ti in 0.1 M oxalic acid with RCCs of 73±3%. In contrast, radiolabeling reactions with the ^{45}Ti solution obtained by elution with MeCN / 0.65 M H_2O_2 afforded much lower RCCs of only 9±6% (maybe due to partial elution of the hydroxamate functional groups from the ZR Resin™).

Conclusions

In this work, a method for the separation of ^{45}Ti from irradiated Sc targets based on column chromatography has been developed and optimized. Using hydroxamate-functionalized ZR Resin™, ^{45}Ti was recovered in yields of 61±8% within 8 min resulting in an overall time of 15 min for the whole target processing. Contamination of ^{45}Ti with other metals can hamper the radiolabeling process and higher chelator/precursor amounts are necessary. Therefore, the optimized separation process allowed to decrease the final Sc amount from 70 mg/mL to 3.0±1.8 µg/mL. Additionally, the toxicity of the metal has to be taken into account for in vivo applications. Given that Scandium is reported to be a non-toxic element (LD50 > 400 mg/kg) [27] , no adverse effects on toxicity are anticipated., . Subsequent complexation of ^{45}Ti with CDTA afforded [^{45}Ti]Ti(cdta) in RCCs of 73±3%. In terms of its simplicity and short duration, the reported approach is advantageous in comparison with other methods (Table 1), since it is amenable to automation and applicable for the preparation of ^{45}Ti-labeled compounds. The final aim of this separation technique is to obtain at least 50 and 180 MBq for in vivo application. Thus, the in this study achieved activities estimated from a comparison of the amount of ^{44}SC are already sufficient for a single PET examination. .

Declarations

The authors have no conflicts of interest to declare that are relevant to the content of this article.

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

References

1. Kostelnik TI, Orvig C (2019) Radioactive Main Group and Rare Earth Metals for Imaging and Therapy. Chem Rev 119:902–956. https://doi.org/10.1021/acs.chemrev.8b00294
2. Roesch F, Martin M (2023) Radiometal-theranostics: the first 20 years*. J Radioanal Nucl Chem 332:1557–1576. https://doi.org/10.1007/s10967-022-08624-3
3. Welch MJ, Laforest R, Lewis JS (2006) Production of Non-standard PET Radionuclides and the Application of Radiopharmaceuticals Labeled with these Nuclides. In: Schubiger PA, Lehmann L, Friebe M (eds) PET Chemistry The Driving Force in Molecular Imaging. Springer, Heidelberg, pp 159–182
4. Qaim SM, Spahn I (2018) Development of novel radionuclides for medical applications. J Labelled Comp Radiopharm 61:126–140. https://doi.org/10.1002/jlcr.3578
5. Qaim SM, Scholten B, Neumaier B (2018) New developments in the production of theranostic pairs of radionuclides. J Radioanal Nucl Chem 318:1493–1509. https://doi.org/10.1007/s10967-018-6238-x
6. Qaim SM (2008) Decay data and production yields of some non-standard positron emitters used in PET. The quarterly journal of nuclear medicine and molecular imaging : official publication of the Italian Association of Nuclear Medicine (AIMN) [and] the International Association of Radiopharmacology (IAR), [and] Section of the Society of. 52:111–20
7. Notni J, Wester HJ (2018) Re-thinking the role of radiometal isotopes: Towards a future concept for theranostic radiopharmaceuticals. J Labelled Comp Radiopharm 61:141–153. https://doi.org/10.1002/jlcr.3582
8. Martiniova L, De Palatis L, Etchebehere E, Ravizzini G (2016) Gallium-68 in Medical Imaging. Curr Radiopharm 9:187–207. https://doi.org/10.2174/1874471009666161028150

9. Walczak R, Krajewski S, Szkliniarz K, et al (2015) Cyclotron production of 43Sc for PET imaging. EJNMMI Phys 2:1–10. https://doi.org/10.1186/s40658-015-0136-x
10. Domnanich KA, Eichler R, Müller C, et al (2017) Production and separation of 43Sc for radiopharmaceutical purposes. EJNMMI Radiopharm Chem 2:1–17. https://doi.org/10.1186/s41181-017-0033-9
11. Qaim SM (2011) Development of novel positron emitters for medical applications: nuclear and radiochemical aspects. ract 99:611–625. https://doi.org/10.1524/ract.2011.1870
12. Costa P, Metello L, Alves F, Duarte Naia M (2018) Cyclotron Production of Unconventional Radionuclides for PET Imaging: the Example of Titanium-45 and Its Applications. Instruments 2:8. https://doi.org/10.3390/instruments2020008
13. Pedersen KS, Imbrogno J, Fonslet J, et al (2018) Liquid-liquid extraction in flow of the radioisotope titanium-45 for positron emission tomography applications. React Chem Eng 3:898–904. https://doi.org/10.1039/c8re00175h
14. Giesen K, Spahn I, Neumaier B (2020) Thermochromatographic separation of 45Ti and subsequent radiosynthesis of [45Ti]salan. J Radioanal Nucl Chem 326:1281–1287. https://doi.org/10.1007/s10967-020-07376-2
15. Chen F, Valdovinos HF, Hernandez R, et al (2017) Intrinsic radiolabeling of Titanium-45 using mesoporous silica nanoparticles. Acta Pharmacol Sin 38:907–913. https://doi.org/10.1038/aps.2017.1
16. Severin GW, Nielsen CH, Jensen AI, et al (2015) Bringing Radiotracing to Titanium-Based Antineoplastics: Solid Phase Radiosynthesis, PET and ex Vivo Evaluation of Antitumor Agent [45Ti](salan)Ti(dipic). J Med Chem 58:7591–7595. https://doi.org/10.1021/acs.jmedchem.5b01167
17. Gagnon K, Severin GW, Barnhart TE, et al (2012) [sup 45]Ti extraction using hydroxamate resin. In: AIP Conference Proceedings. pp 211–214
18. Chaple IF, Thiele K, Thaggard G, et al (2020) Optimized methods for production and purification of Titanium-45. Applied Radiation and Isotopes 166:109398. https://doi.org/10.1016/j.apradiso.2020.109398

19. Vavere AL, Jones LA, Mccarthy TJ, et al (2001) Preparation, biodistribution, and micropet imaging of 45Ti-transferrin. J Labelled Comp Radiopharm 44:S793–S795. https://doi.org/10.1002/jlcr.25804401279
20. Koller AJ, Wang L, Deluca M, et al (2023) De Novo Approaches to the Solid-Phase Separation of Titanium(IV) and Scandium(III): Translating Speciation Data to Selective On-Bead Chelation toward Applications in Nuclear Medicine. Inorg Chem. https://doi.org/10.1021/acs.inorgchem.3c01493
21. Severin GW, Nielsen CH, Jensen AI, et al (2015) Bringing Radiotracing to Titanium-Based Antineoplastics: Solid Phase Radiosynthesis, PET and ex Vivo Evaluation of Antitumor Agent [45Ti](salan)Ti(dipic). J Med Chem 58:7591–7595. https://doi.org/10.1021/acs.jmedchem.5b01167
22. Chaple IF, Lapi SE (2018) Production and Use of the First-Row Transition Metal PET Radionuclides 43,44Sc, 52Mn, and 45Ti. Journal of Nuclear Medicine 59:1655–1659. https://doi.org/10.2967/jnumed.118.213264
23. Vavere AL, Welch MJ (2005) Preparation, biodistribution, and small animal PET of 45Ti- transferrin. Journal of Nuclear Medicine 46:683–690
24. Radchenko V, Meyer CAL, Engle JW, et al (2016) Separation of 44Ti from proton irradiated scandium by using solid-phase extraction chromatography and design of 44Ti/44Sc generator system. J Chromatogr A 1477:39–46. https://doi.org/10.1016/j.chroma.2016.11.047
25. Triskem PRODUCT SHEET ZR Resin. https://www.triskem-international.com/scripts/files/61eef0f5e9fa69.45400285/PS_ZR-Resin_EN_210908.pdf. Accessed 23 Aug 2023
26. Herth MM, Ametamey S, Antuganov D, et al (2021) On the consensus nomenclature rules for radiopharmaceutical chemistry – Reconsideration of radiochemical conversion. Nucl Med Biol 93:19–21. https://doi.org/10.1016/j.nucmedbio.2020.11.003
27. Weng W, Biesiekierski A, Li Y, et al (2021) A review of the physiological impact of rare earth elements and their uses in biomedical Mg alloys. Acta Biomater 130:80–97. https://doi.org/10.1016/j.actbio.2021.06.004

28. Dietlein M, Kobe C, Kuhnert G, et al (2015) Comparison of [18F]DCFPyL and [68Ga]Ga-PSMA-HBED-CC for PSMA-PET Imaging in Patients with Relapsed Prostate Cancer. Mol Imaging Biol 17:575–584. https://doi.org/10.1007/s11307-015-0866-0

29. Eppard E, de la Fuente A, Benešová M, et al (2017) Clinical Translation and First In-Human Use of [^{44} Sc]Sc-PSMA-617 for PET Imaging of Metastasized Castrate-Resistant Prostate Cancer. Theranostics 7:4359–4369. https://doi.org/10.7150/thno.20586

30. Liu Q-X, Zhou Z-H (2012) Monomeric peroxo titanate coordinated with cyclohexanediaminetetraacetate: Towards the active oxygen species of the Ti(IV) site hosted in the titanium silicalite catalyst TS-1. Polyhedron 35:1–6. https://doi.org/10.1016/j.poly.2011.12.037

Supplementary information

Materials and Methods

1. Chemicals and Materials:

All chemicals were used without further purification. Hydrochloric acid (analytical grade) was purchased from TH.GEYER (Renningen, Germany). Type 1 ultra-pure water (≥18 MΩ cm) was prepared onsite with a Purelab classic water purification system from ELGA Labwater (Celle, Germany). Zirconium "ZR" resin (containing hydroxamate functional groups) was provided by Triskem Intl. (Bruz, France). Dry dimethylformamide (DMF), *N*,*N*-diisopropylethylamine (DIPEA) (99.5%), 1,2-cyclohexanedinitrilotetraacetic acid (CDTA) (98%), citric acid, tartaric acid, oxalic acid and phosphate salts for buffering were purchased from Sigma-Aldrich (Darmstadt, Germany). The scandium disks (Sc purity TREM > 99.99%) for irradiations were purchased from Smart Elements GmbH (Vienna, Austria). and were supplied as a scandium ingots, which were rolled to plates (thickness: 0.65±0.05 mm, diameter: 13 mm, mass: 330±30 mg). Chlorine gas (5.0) was obtained from Linde Gas (Germany).

Mass flow controllers were supplied from Bronkhorst Deutschland Nord GmbH (Germany) (EL FLOW Select 300 mL/min for inert gases, LOW-ΔP-FLOW 60 mL/min for chlorine gas). Mass flow conversions were done using the Fluidat database (Bronkhorst Nord GmbH, Kamen, Germany). Glassware for the separation system was manufactured by the Central Institute of Engineering, Electronics and Analytics (ZEA-1) at Forschungszentrum Jülich. For thin layer chromatography (TLC), RP silica coated aluminum TLC plates from Sigma-Aldrich (Darmstadt, Germany) were used. The analysis of the radio-TLCs was performed with a PerkinElmer Cyclone Plus Storage Phosphor System (Waltham, MA, USA). Measurement of radioactivity were performed with a DOSE Calibrator TALETE HC (COMECER S.p.A., Castel Bolognese (RA), Italy).

Gamma-ray spectroscopy was performed with ORTEC HPGe spectrometers (AMETEK GmbH, Germany), which were energy and efficiency calibrated with certified radiation

point sources (Co-60, Ba-133, Eu-152, Ra-226) from the Physikalisch-Technische Bundesanstalt (Germany).

Radio-HPLC was performed on a HPLC system consisting of an Azura P 4.1 s pump with an Azura UVD 2.1 s UV/VIS detector (Knauer Wissenschaftliche Geräte GmbH, Germany) and an EG & G Ortec ACE NaI(Tl) radioactivity detector with photomultiplier (EG & G Ortec, USA). The radioactivity detection limit was determined by serial dilution and amounted to 0.3 kBq. Co-elution experiments of radioactive and non-radioactive complexes were performed at a flow rate of 0.7 mL/min using H_2O/PBS/CH_3COOH (96.4/3.1/0.5, v/v/v) as the mobile phase and Synergi Polar-RP 4 µ RP 80 Å, 250 × 4.6 mm (Phenomenex Inc., Germany) as the RP stationary phase. The UV detection wavelength for all measurements was 210 nm.

NMR spectra were measured on a Varian Inova 400 spectrometer (Agilent Technologies, Germany) with 400.1 MHz (^{1}H-NMR) and 100.62 MHz (^{13}C-NMR).

The electrospray ionization (ESI) source was operated in the positive mode. Low-resolution mass spectrometry was performed using a Finnigan Automass Multi spectrometer (Thermoquest, Germany).

2. Target dissolution:

The irradiated target was placed in a 20 mL screw lid jar and cooled in an ice bath. 10 M hydrochloric acid (5 mL) was then added, the jar was closed after 1 min, and the solution was stirred for 5-10 min until the color turned from black to slightly yellow. The resulting solution, which contained around 250 MBq ^{45}Ti, was directly used for the batch experiments or diluted to 20 mL with 10 M hydrochloric acid for further evaluation experiments.

3. Separation experiments

All statistical calculations are in the form MEAN±SD. Decay corrected (d.c.) values are reffered to the start of the separation.

3.1.Preparation and conditioning of resin:

For the separation experiments, chromabond columns (1 mL, equipped with two frits) were filled with ZR Resin™ (66±6 mg, 130±6 mg or 280±6 mg as indicated) which was slurred in water. The columns were conditioned with 10 M HCl (5 mL) and directly used for the separation experiments.

3.2.Preparation of stock solutions:

Table 2: Buffer solutions.

No.	pH	c [mol/L]	Buffer
I	8.0	0.100	NaH_2PO_4 / Na_2HPO_4
II	8.0	1.000	NaH_2PO_4 / Na_2HPO_4
III	8.0	0.067	NaH_2PO_4 / Na_2HPO_4
IV	5.5	0.100	NaH_2PO_4 / Na_2HPO_4
V	5.5	0.500	NaH_2PO_4 / Na_2HPO_4
VI	5.5	1.000	NaH_2PO_4 / Na_2HPO_4
VII	3.3	0.100	HCOOH / NH_3

Table 3: Elution media with respective concentration, pH value and ^{45}Ti recovery d.c.

No.	Media	c [mol/L]	Buffer	pH	^{45}Ti recovery d.c.[%]
1	30% MeOH / H_2O / H_2O_2	0.065	–	–	12.5±2.6
2	30% MeCN / H_2O / H_2O_2	0.065	–	–	17.8±2.2
3	Tartaric acid	0.10	–	–	28.5±5.0
4	1 M HCl / H_2O_2	0.065	–	–	30.8±3.3
5	Citric acid	0.10	–	–	35.3±5.3
6	0.65 M H_2O_2	0.65	–	–	35.0±13.3
7	Oxalic acid	0.50	–	–	59.1±24.0
8	MeCN	–	–	–	72.3±10.3
9	MeCN / H_2O_2	0.65	–	–	82.3±7.4
10	Oxalic acid	0.10	–	1.3	28.9±6.0
11	Oxalic acid	0.05	–	1.5	13.9
12	Oxalic acid	0.05	IV	2.6	36.2±4.6
13	Oxalic acid	0.05	VII	2.8	33.4±14.7
14	Oxalic acid	0.01	VII	3.2	5.3
15	Oxalic acid	0.10	V	3.8	16.9
16	Oxalic acid	0.05	I	5.1	8.6
17	Oxalic acid	0.10	VI	5.3	9.9
18	Oxalic acid	0.10	II	7.0	8.1
19	Oxalic acid	0.01	II	7.9	1.9
20	Oxalic acid	0.10	III	2.8	65.2±1.2
21	Oxalic acid	0.20	III	2.8, adjusted with NaOH	66.6±6.1
22	20% MeOH / Oxalic acid	0.1	III	2.8	60.8±5.9

3.3.Eluent screening:

Aliquots (1 mL) of the ^{45}Ti target solution were applied to a series of columns filled with 130±6 mg ZR Resin™ and each column was washed with 10 M HCl (5 mL) and H_2O (5 mL). Afterwards, 2.5 mL of the eluents No. 1-9 (Table 2) were used to elute the ^{45}Ti and the percentage of ^{45}Ti in the resulting solutions was determined. The ^{45}Ti recoveries observed with the different eluents (n=3 per eluent) are summarized in Table 2.

3.4.pH screening

Aliquots (1 mL) of the ^{45}Ti target solution were applied to a series of columns filled with 130±6 mg ZR Resin™ and each column was washed with 10 M HCl (5 mL) and H_2O (5 mL). Afterwards, 2.5 mL of the eluents No. 10-19 (Table 2) were used to elute the ^{45}Ti and the percentage of ^{45}Ti in the resulting solutions was determined. The ^{45}Ti recoveries observed with the different eluents (n=1-3 per eluent, as indicated) are summarized in Table 2.

3.5.Further oxalic acid eluents

Aliquots (1 mL) of the ^{45}Ti target solution were applied to a series of columns filled with 130±6 mg ZR Resin™ and each column was washed with 10 M HCl (5 mL) and H_2O (5 mL). Afterwards, 2.5 mL of the eluents No. 20-22 (Table 2) were used to elute the ^{45}Ti and the percentage of ^{45}Ti in the resulting solutions was determined (n=3 per eluent).

3.6.Minimization of Sc content

For this experiment, the Sc target was irradiated without degrader foil to enable use of the co-produced ^{44}Sc as a radiotracer for determination of the Sc content. Aliquots (1 mL) of the ^{45}Ti/^{44}Sc target solution were applied to a series of columns filled with 130±6 mg ZR Resin™ and the columns were

- not washed,
- washed with 5 mL of H_2O,
- washed with 5 mL of 10 M HCl and an equal volume of H_2O,

- washed with 10 mL of 10 M HCl and an equal volume of H_2O,
- washed with 15 mL of 10 M HCl and an equal volume of H_2O.

Afterwards, 2.5 mL of 0.1 M oxalic acid (pH 2.8) was used to elute the ^{45}Ti and ^{44}Sc and the resulting solutions were analyzed by γ-ray spectroscopy. The activity of ^{45}Ti (E_γ=719.6 keV, I_γ=0.154% and E_γ=511 keV) and ^{44}Sc (E_γ=1157.02 keV, I_γ=94.3%) from each solution was calculated and the ^{45}Ti:^{44}Sc ratio were calculated. The ^{45}Ti:^{44}Sc ratios observed with the different wash volumes (n=3 per volume) are summarized in Table 4.

Table 4: ***^{45}Ti/^{44}Sc-ratio obtained with different volumes of the wash solutions.***

	^{45}Ti/^{44}Sc
Target solution	20±11
No washing step	36±2
5 mL H_2O	380±80
5 mL HCl / 5 mL H_2O	4000±300
10 mL HCl / 10 mL H_2O	3600±200
15 mL HCl / 15 mL H_2O	130±40

3.7.Batch experiment

A) Determination of Sc-contamination

Columns filled with 130±6 mg ZR Resin™ were prepared, a non-irradiated target solution (350 mg in 4 mL 10 M HCl) was transferred to the column, and the column was washed with 10 M HCl (5 mL) and H_2O (5 mL). Afterwards, 0.1 M oxalic acid (pH = 2.8) was used for elution. For the first experiment a total of 2.5 mL of 0.1 M oxalic acid was used and 0.5 mL fractions were collected. Each fraction was analyzed by ICP-MS. This resulted in the elution profile for Sc. Then two further experiments were done with the same conditions and 2.5 mL 0.1 M oxalic acid was used for elution. The solutions were analyzed by ICP-MS. The results of the conducted experiments are given in Table 5. The average Sc-contamination was determined from the sum of the elution profile and the two experiments (3.0±1.8 mg/mL).

Table 5: Results of the ICP-MS analysis of Sc in the final solution**.**

	V [mL]	**ρ(Sc) [µg/mL]**
1	0.5	0.113±0.012
2	0.5	0.797±0.017
3	0.5	0.182±0.009
4	0.5	0.182±0.009
5	0.5	0.021±0.009
1-5	2.5	1.27
7	2.5	4.84±0.09
8	2.5	2.86±0.04

B) Batch experiments with aliquots

Columns filled with 130±6 mg ZR Resin™ were prepared, aliquots (1 mL) of the target solution (6-35 MBq) were transferred to the columns, and the columns were washed with 10 M HCl (5 mL) and H_2O (5 mL). Afterwards, 2.5 mL 0.1 M oxalic acid (pH = 2.8) was used to elute the ^{45}Ti. The results are summarized in Table 5.

Table 6: ^{45}Ti recovery with optimized conditions.

A_0 [MBq]	**^{45}Ti Recovery [MBq]**	**^{45}Ti Recovery d.c. [%]**	**A_0 [MBq]**	**^{45}Ti Recovery [MBq]**	**^{45}Ti Recovery d.c. [%]**
10.22	7.55	88.46	9.58	4.82	61.61
7.1	3.86	59.06	5.68	3.04	73.69
5.96	3.28	61.38	7.44	3.6	64.26
18.25	14.11	86.04	12.24	3.96	62.32
13.69	8.4	66.9	6.54	4.03	70.09
16.7	10.7	71.43	5.67	2.35	67.77
35.24	22.9	71.48	5.39	2.6	54.19
10.1	5.99	78.6	11.45	7.63	72.82
10.2	4.89	53.83	10.63	7.14	74.24
7.99	5.6	76.1	8.69	5.66	80.15
7.14	4.64	77.6	7.27	5.13	78.3
11.15	4.97	52.09	6.37	2.9	51.42

C) Scaled up experiments with whole target

Columns filled with 130±6 mg ZR Resin™ were prepared, a complete target solution (Sc: 330±30 mg, ^{45}Ti: 107-180 MBq, V=5 mL) was transferred to the columns, and the columns were washed with 10 M HCl (5 mL) and H_2O (5 mL). Afterwards, 2.5 mL 0.1 M oxalic acid (pH = 2.8) was used to elute the ^{45}Ti. The results are summarized in Table 6.

Table 7: Scaled-up experiment. ^{45}Ti recovery and separation time**.**

A_0 [MBq]	^{45}Ti Recovery [MBq]	^{45}Ti Recovery d.c. [%]	t [min]
176	120	69.21	4
120	84	71.05	5
160	75	47.76	5
178	102	58.16	4
124	85	69.84	4
137	84	62.23	9
114	63	56.72	11
107	64	61.85	8
140	83	61.54	10
146	75	53.12	9

3.8.Determination of elution profile

Columns filled with 130±6 mg ZR Resin™ were prepared, aliquots (1 mL) of the target solution (21-29 MBq) were transferred to the columns, and the columns were washed with 10 M HCl (5 mL) and H_2O (5 mL). Afterwards, 0.1 M oxalic acid (pH 2.8) (n=3) were used to elute the ^{45}Ti and the respective eluate was collected in 0.5 mL fractions.

4. Ti(cdta)

4.1. Synthesis of Ti(cdta)(H_2O)

Under an argon atmosphere, 1,2-cyclohexanedinitrilotetraacetic acid (CDTA) (730 g, 2.1 mmol, 1.00 eq.) was dissolved in dry DMF (5 mL) and $TiCl_4(thf)_2$ (700 mg, 2,1 mmol, 1.00 eq.) was added to the solution. After stirring for 45 min at 70 °C, the organic solvent was removed under reduced pressure and the crude product was recrystallized from water. The product was obtained as colorless crystals (165 mg, yield: 20%). A single crystal was analyzed by x-ray diffraction and the results were in accordance with those from Liu et al. [30] .

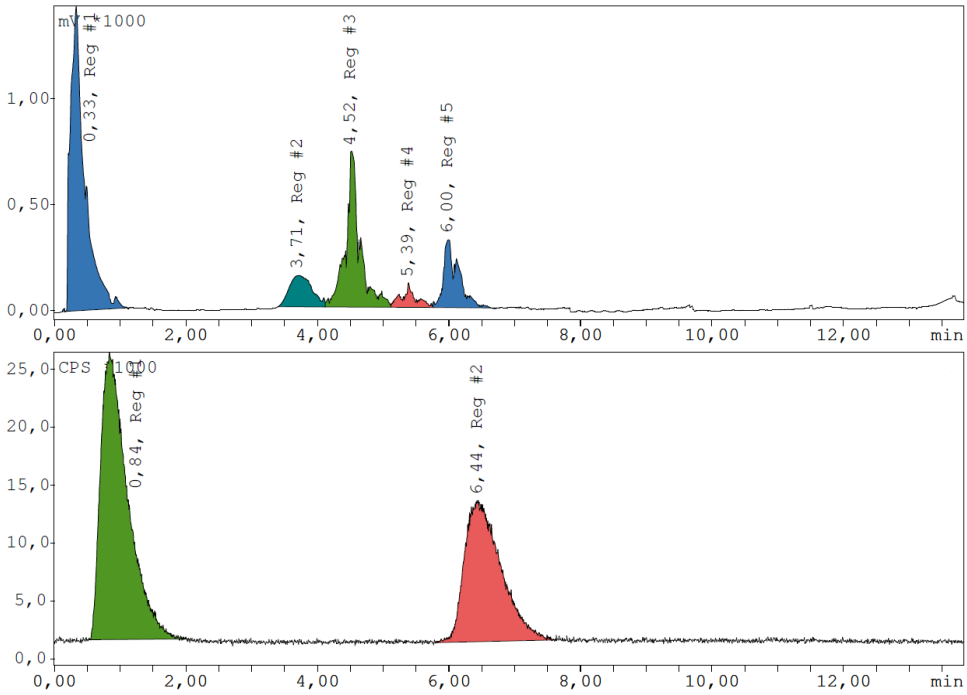
^{1}H-NMR (400 MHz, DMSO) δ 4.21 (d, *J* = 17.3 Hz, 2H), 3.90 – 3.74 (m, 4H), 3.65 – 3.58 (m, 2H), 3.19 (d, *J* = 8.0 Hz, 2H), 1.93 (d, *J* = 12.1 Hz, 2H), 1.67 (d, *J* = 8.0 Hz, 2H), 1.42 (d, *J* = 3.7 Hz, 2H), 1.17 (t, *J* = 9.6 Hz, 2H).

^{13}C-NMR (101 MHz, DMSO) δ 174.36 (s), 173.32 (s), 67.21 (s), 65.24 (s), 58.22 (s), 53.40 (s), 41.67 (s), 25.08 (s), 23.35 (s), 18.04 (s), 16.75 (s), 12.29 (s).

4.2. Complexation of ^{45}Ti using CDTA

[^{45}Ti]$TiCl_4$ (thermochromatography):

[^{45}Ti]$TiCl_4$ was obtained by thermochromatography using a previously reported method [5] and dissolved in dry THF (81 MBq in 2 mL). For radiolabeling, CDTA (1 mg) and DIPEA (20 µL) in anhydrous DMF (2mL) were added to a reaction vessel containing the isolated [^{45}Ti]$TiCl_4$ in THF and the mixture was allowed to react for 45 min at room temperature. The reaction mixture was then quenched by addition of H_2O (0.5 mL) and analyzed by HPLC (Hydro-RP: H_2O/PBS/EtOH 96.4/3.1/0.5, 0.7 mL/min) and radio-TLC (RP: 90% H_2O / 10% MeCN, R_f=0.85).



SI-Fig. 1: Chromatogram of [^{45}Ti]Ti(cdta) co-injected with the non-radioactive reference compound Ti(cdta). Top UV-channel: Post column injection (0.33 min), solvents DMF/THF (3.71/4.52 min), CDTA (5.39 min), Ti(cdta) 6.00 min. Bottom radioactivity channel: Post column injection (0.84 min), [^{45}Ti]Ti(cdta) (6.44 min).

^{45}Ti-containing 0.65 mM H_2O_2 in MeCN solution (column chromatography):

Columns filled with 130±6 mg ZR Resin™ were prepared, aliquots (1 mL) of the target solution (6-35 MBq) were transferred to the columns, and the columns were washed with 10 M HCl (5 mL) and H_2O (5 mL). Afterwards, *0.65 mM H_2O_2 in MeCN* (n=3) were used to elute the ^{45}Ti. CDTA (1 mg) was dissolved in sodium phosphate buffer (pH = 8.0, 300 µL), the solution was added to a reaction vessel containing the isolated ^{45}Ti. The mixture was allowed to react for 45 min at room temperature, after which it was analyzed by radio-TLC.

^{45}Ti-oxalate solution (column chromatography):

CDTA (1 mg) was dissolved in sodium phosphate buffer (pH = 8.0, 300 µL), the solution was added to a reaction vessel containing the isolated [^{45}Ti]Ti-oxalate complex, and the

mixture was allowed to react for 45 min at room temperature, after which it was analyzed by radio-TLC.