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Separation of radioactive terbium from massive Gd targets for medical use

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Abstract
Methods for chemical separation of terbium from gadolinium and dysprosium have been investigated using proton activated gadolinium, as well as gadolinium–terbium mixture matrix. Terbium radioisotopes can be effectively separated from large amount (> 100 mg) gadolinium on an analytical column. In reactors inactive dysprosium also forms in the activated Gd matrix, considerably decreasing the chemical purity of the labelled radiopharmaceutical. A semi-preparative column method was therefore developed to separate terbium at the same time both from the dysprosium and the gadolinium target material.

Keywords Terbium · Gadolinium · Dysprosium · Proton irradiation · Ion-exchange · Chemical separation

Introduction

It is well known that cancer is responsible for more than 20% of deaths worldwide. Targeting cancerous sites with therapeutic proteins or peptides has become an effective and important tool for therapy [1, 2]. Combining (or labeling) these target vectors with certain radioactive isotopes could increase their therapeutic efficacy. Nowadays several radioisotopes are used for labeling of these biomolecules and more are under research. Some radioisotopes have big advantages both for diagnostic and cancer therapy. In diagnostic application they offer a non-invasive method to find tumors in the human body, mainly with PET or SPECT techniques. They are also useful in therapy, because they increase the efficacy of the target vectors via destroying tumors with their decay energy (radiation dose). It is also known as "cross-fire effect". These are the main reasons why they are in the center of research for medical application.

Terbium is a special element since it offers radioisotopes suitable for PET, SPECT diagnosis and α and β^- therapy

as well. Due to this versatility, terbium is often called the "Swiss Army knife" of radioisotopes [3, 4]. The medically important four terbium radioisotopes are shown on Table 1. All of these radionuclides possess one or several advantages compared to radionuclides currently used or under investigation in radiopharmacology with respect to decay energies and half-life:

(a) For diagnostic imaging the ^{155}Tb seems to be an ideal nuclide [5, 6]. Its half-life of 5.3 days is long enough to observe even slower metabolic processes. The characteristic γ-rays at 87 and 105 keV are ideal for most SPECT cameras.

(b) For quantitative imaging with PET the ^{152g}Tb seems to be promising [4]. The positron intensity of 17% and the mean positron energy of 1.1 MeV are suitable. The only strong γ line of 344 keV ($I_\gamma = 65\%$) is far from the annihilation line of 511 keV to avoid coincidence events.

(c) For targeted particle therapy both the ^{149}Tb ($T_{1/2} = 4.1$ h, $I_\alpha = 17\%$) and ^{161}Tb ($T_{1/2} = 6.9$ days, $I_{\beta^-} = 100\%$) seems to be promising. ^{161}Tb has a similar half-life and β energy to the already used ^{177}Lu [7].

These isotopes can be produced with particle accelerator [3, 4, 6, 8, 9] or in nuclear reactors [7, 10, 11]; depending on which isotope is necessary for the given application. Several of the possible production routes of terbium

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Table 1 Terbium radioisotopes useful in nuclear medicine and their decay properties [16]

Nuclides	Half-life	Decay mode	γ-rays (keV)	Intensity (%)
^{149}Tb	4.118 h	$\varepsilon + \beta^+$: 83.3%	352.24	29.4
		α: 16.7%		
^{152}Tb	17.5 h	$\varepsilon + \beta^+$: 100%	271.13	8.6
		β^+: 17%	344.27	65.0
			586.26	9.4
^{155}Tb	5.32 days	ε: 100%	180.10	7.5
			367.23	1.5
^{161}Tb	6.89 days	β^-: 100%	25.65	23.2
			48.91	17.0
			57.19	1.8
			74.57	10.2

radioisotopes from gadolinium, including proton and neutron activation, are shown on Fig. 1. If a cancerous site is found in the human body by using a target vector labeled with a diagnostic terbium radioisotope, it can be simply replaced to a therapeutic Tb one in the radiopharmaceutical, ensuring the same biochemical route.

Despite of the opportunity, only a few in vivo applications of Tb-radionuclide labeled target vectors are reported, particularly using ^{152}Tb [12]. It is probably due to the lack of well established production and separation methods

[5, 13]. Majority of literature sources report separation of microgram amounts of rare earth from each other. Only few groups investigated the separation of ^{161}Tb from large amount of Gd targets, irradiated in reactor, while we could not find any reported results for cyclotron irradiated massive natural Gd target. Tiung and co-workers published their results for separation of ^{161}Tb radioisotopes using extraction chromatography [14], while van der Meulen and co-workers carried out ion exchange separation on semi-preparative column [15].

In the case of neutron activation of Gd, inactive dysprosium (see Fig. 1) is also co-formed. After long activation, the amount of the produced Dy, having very similar chemical characteristics as Tb, could considerably decrease the chemical purity of the labelled radiopharmaceutical causing problems in the imaging procedure [10].

The aim of the present work was to develop new, more effective Tb separation methods from massive (> 100 mg) Gd targets using (1) an analytical HPLC column and (2) a semi-preparative column for the concurrent separation of Tb and Dy from Gd.

Experimental

For the experiments where we wanted to compare the separation efficiency of the analytical HPLC and the semi-preparative column methods, we have prepared targets

Fig. 1 Terbium radioisotopes production routes from gadolinium
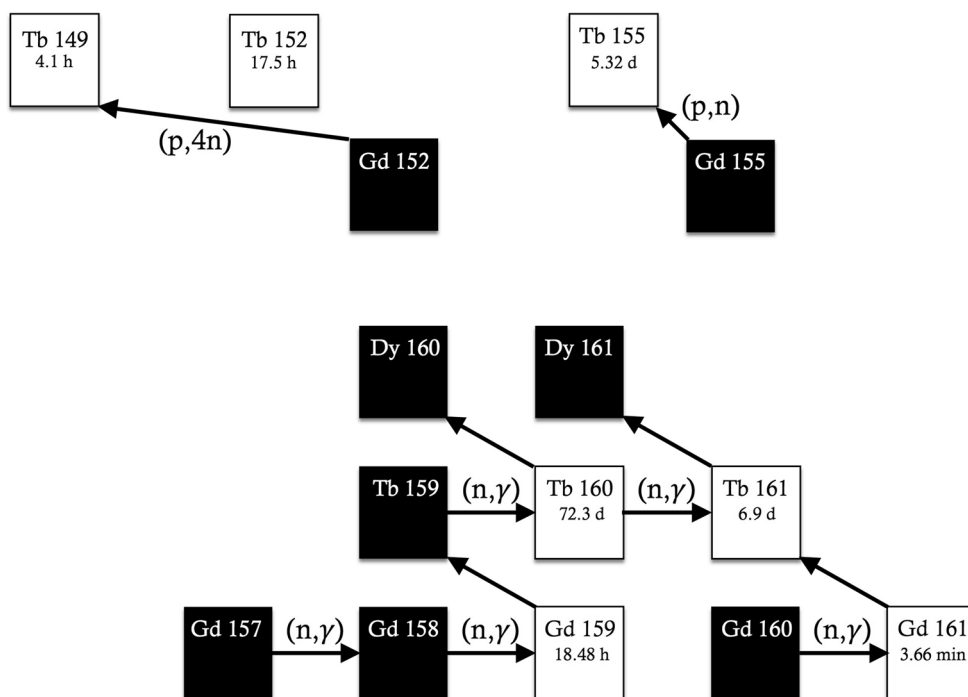
using gadolinium of natural isotopic composition in pressed tablets form of gadolinium-oxide (Sigma-Aldrich) with purity of 99.99%. These targets contained either 175 or 350 mg gadolinium.

In the second case (Dy experiment) we made pressed tablets using equal amount (175 mg each) of gadolinium-oxide and terbium-oxide (Sigma-Aldrich) in order to get a mixture of Tb, Gd and Dy radioisotopes after target activation.

Targets were irradiated with our MGC-20 cyclotron for 3 h (E_p = 15 MeV, I_p = 50 nA). These parameters were selected based on our cross section measurements published in our previous report [8]. The ^{159}Dy [^{159}Tb(p,n)^{159}Dy] and ^{156}Tb [^{156}Gd(p.n)^{156}Tb] isotopes were used for indication of dysprosium and terbium in our separated samples, and the ^{159}Gd [^{160}Gd(p,pn)^{159}Gd] radioisotope was used for tracing the gadolinium.

The irradiated targets were dissolved in cc. HCl (WVR, AnalaR Normapur Reag. Ph. Eur.) and evaporated to dryness. The residues were re-dissolved in 0.5 ml (for experiment on analytical column) and in 2 ml (for semi-preparative experiment) of 0.05 M NH_4Cl (Reanal) solution before the separation. For separation BioRad AG50W-X8 (mesh size 200–400) cation exchange resin was used with α-hydroxyisobutyric acid (α-HIBA, Sigma-Aldrich) eluent. Some literature sources suggest that the α-HIBA elutes the elements with different retention time depending on the concentrations [10, 11]. 0.14 M α-HIBA was applied for dysprosium, 0.2 M for terbium and 0.5 M for gadolinium elution. The pH of the eluents was adjusted to 4.5 with NH_4CO_3 (Reanal). 200 × 5 mm column (GE) was used for the experiments on an analytical column, and 110 × 15 mm column (BioRad) was used in the case of semi-preparative experiments. The columns were filled with cation exchange resin what was preliminary treated to obtain Na^+ form. The columns were connected to a HPLC pump (Gilson). The dissolved targets were loaded into an injection loop and then were loaded onto the columns with 0.5 ml/min flow rate. The columns were washed with one column volume equivalent water after the loading. The separations were carried out by isocratic elution with 1.0 or 0.6 ml/min flow rate.

The elution was followed up with the HPLC's in-line γ-counter (Biomed). Mainly 2 ml fractions were collected manually during the elution. After the separation processes, the collected fractions were measured with Canberra HPGe γ-spectrometer. The samples were measured until the uncertainty of the peak area of the measured γ-line reduced below 5%. The quantitative isotopic contents were determined by using the 534.32 keV γ-line for the terbium (^{156}Tb), 363.55 keV for gadolinium (^{159}Gd) and 58.00 keV for dysprosium (^{159}Dy).

Each Tb fraction (around 40 ml, see Discussion) has to be purified from the α-HIBA before using for labeling. For this purpose each separated fraction was diluted with 1 M HCl in order to adjust the pH around 1. This solution was passed through a small (40 × 8 mm) column (BioRad) filled with the same resin what we used above for separation. 0.6 ml/min flow rate was used for the processes. The column was washed with five column volume equivalent 1 M HCl to eliminate the α-HIBA from the column. The Tb was completely eluted with 4 M HCl [10]. The same procedure can be used if recovery of Gd is desired, i.e., when enriched Gd target is used.

Results and discussion

Tb/Gd separation on an analytical column

Separation of radioterbium from 175 to 350 mg irradiated Gd target was studied on an analytical column with both 1.0 and 0.6 ml/min elution speed. In all the four separation processes very similar results were received. In Fig. 2 two chromatograms are shown. Curve #A was got from the separation of radioterbium from 175 mg irradiated Gd target at 1.0 ml/min elution speed, while curve #B was obtained with 350 mg irradiated target at 0.6 ml/min. The chromatograms were obtained by γ-measurements of the fractions.

The elutions were started with the 0.2 M α-HIBA and it was continued until the activity dropped to 7% of the maximum of the Tb signal on the HPLC's γ-counter, then it was changed to 0.5 M α-HIBA. The 0.2 M α-HIBA was used in volume of 58 ml for #A and 59 ml for #B experiment. In these cases we got the terbium free from ^{159}Gd isotope when the fractions were collected until 41 ml in #A and 43 ml in #B case.

The presented two curves, as well as the other two, have very similar separation profiles. The separation yield of terbium from the gadolinium, calculated from the measured fractions, is also the same: 85 ± 2%. These results were obtained with a relatively big target weight on an analytical column. Probably this can explain the unusual (proportional) rise at the beginning of the elution peak of massive Gd. Nevertheless, it has no effect for the complete collection of Gd target mass, what is very important in the case of using enriched targets in real production runs.

Fig. 2 Separation of Tb from Gd on analytical column. **#A** 0.2 M α-HIBA and 0.5 M α-HIBA with 1 ml/min, 175 mg target. **#B** 0.2 M α-HIBA and 0.5 M α-HIBA with 0.6 ml/min, 350 mg target
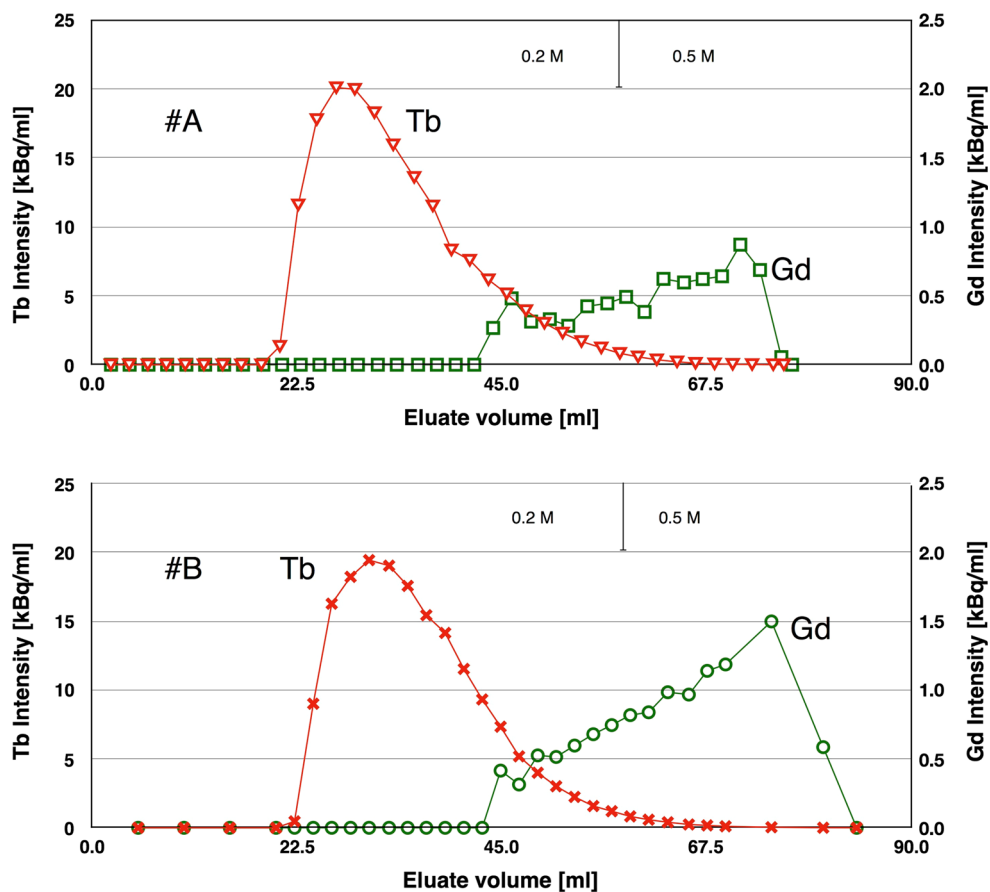

Fig. 3 Separation of Tb from Gd on analytical (**#B**) and semi-preparative (**#C**) columns. **#B** 0.2 M α-HIBA and 0.5 M α-HIBA with 0.6 ml/min, 350 mg target. **#C** 0.2 M α-HIBA and 0.5 M α-HIBA with 0.6 ml/min, 350 mg target
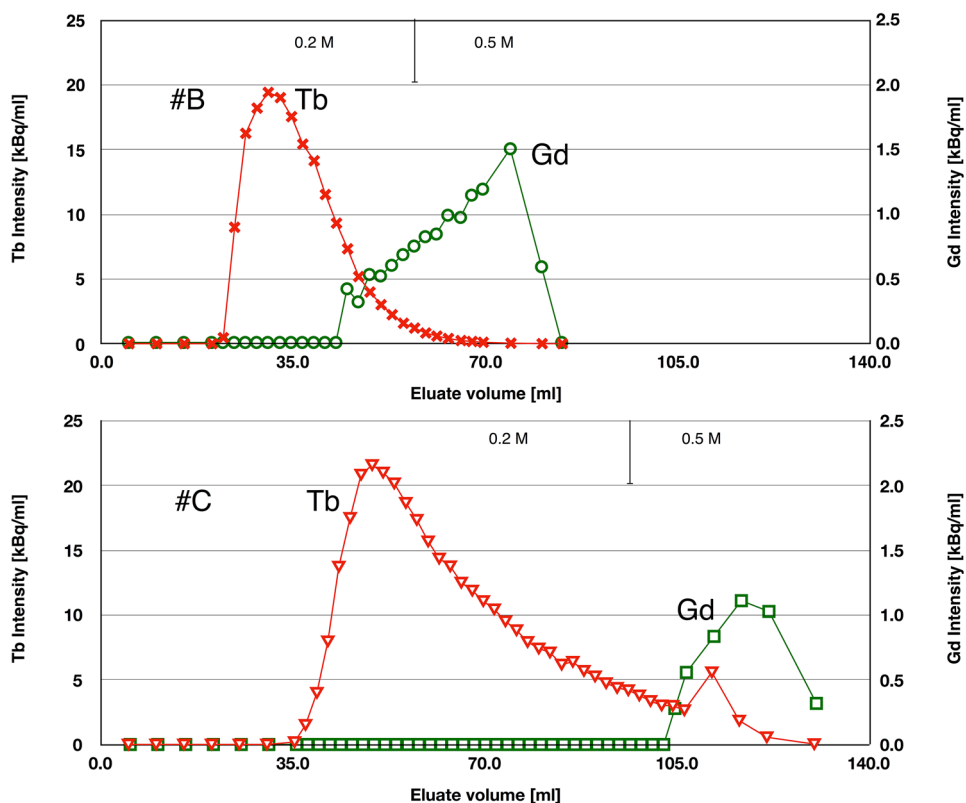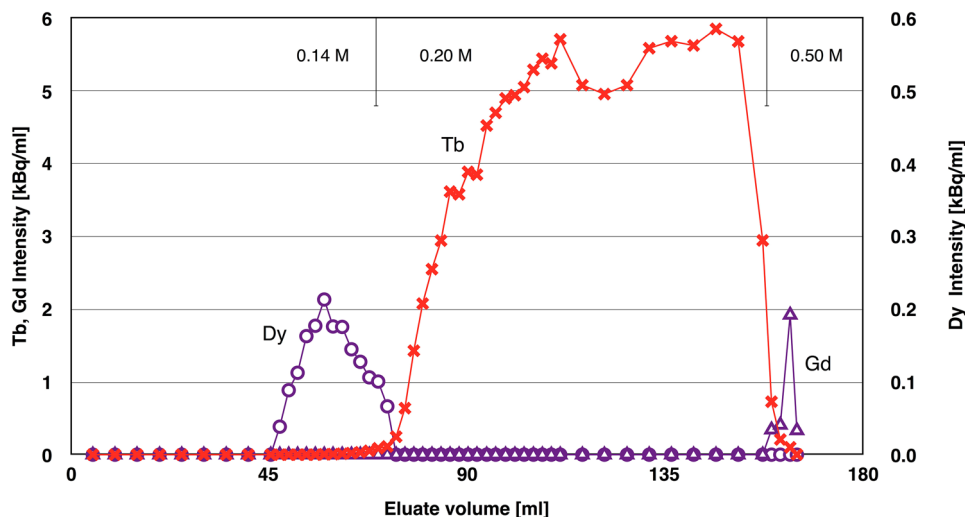
Fig. 4 Simultaneous separation of Tb from Dy and Gd on semi-preparative column. 0.14, 0.2, 0.5 M α-HIBA with 0.6 ml/min, 175 mg Gd$_2$O$_3$ and 175 mg Tb$_2$O$_3$ mixed target

Tb/Gd separation comparison of analytical column and semi-preparative column

The separation was also performed with a semi-preparative column for comparison. The separation was carried out from 350 mg target at 0.6 ml/min elution speed. The bed volume of the semi-preparative column was almost five times bigger than the analytical. Two different eluent concentrations were also used (0.2 and 0.5 M α-HIBA). The shape of the curve #C, obtained on semi-preparative column, are different from the curve #B got on analytical one, as it is presented in Fig. 3. 93 ± 2% of the terbium free from gadolinium could be collected on semi-preparative column.

Although the separation yield on analytical column was 8% lower, this difference is not significant, and can be easily compensated by more beam current or longer irradiation during a real production run.

Tb/Dy separation

As it was mentioned above, in several cases the terbium product has to be separated from the Dy before using for labeling. In this experiment, the separation of terbium from dysprosium and the gadolinium target material was carried out in one procedure.

The result was obtained on a semi-preparative column with 0.6 ml/min elution speed. The composition of the target was 175 mg Gd$_2$O$_3$ and 175 mg Tb$_2$O$_3$. Three different eluent concentrations were used: 0.14 M α-HIBA was applied first followed by 0.2 M and finally with 0.5 M α-HIBA.

In Fig. 4 the separation of Dy and Tb, as well as the Gd fractions are shown. The separation yield of radioterbium, free from ^{159}Dy and ^{159}Gd, was calculated from the measured fractions and reached 99%. Therefore, if dysprosium

is produced during the irradiation, we can simple purify the terbium from both of the contaminants at the same time. In those samples, where the content of the radiogadolinium and radiodysprosium was not detectable by γ-spectrometry, only very small amount Gd and Dy could remain what, nevertheless, requires further ICP-MS control.

Conclusions

A method was developed to separate the medically important radioactive terbium from hundreds of milligrams of gadolinium target on analytical HPLC and semi-preparative columns. The efficiencies of separation techniques were comparable being 85 and 93%, respectively. For reactor irradiations, where dysprosium is also produced from gadolinium, an effective separation method was worked out on semi-preparative column to obtain Dy and Gd free radioactive terbium in one procedure with 99% separation yield. In samples, where the radiogadolinium and radiodysprosium could not be detected with γ-spectrometer within the terbium peak, further measurements have to be carried out to determine the presence of Gd and Dy, if there is any, in the Tb fraction.

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