Rapid Elution of ^{226}Th from a Two-Column ^{230}U/^{226}Th Generator with Diluted and Buffer Solutions

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Article

Rapid Elution of ^{226}Th from a Two-Column ^{230}U/^{226}Th Generator with Diluted and Buffer Solutions

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Abstract: A unique two-column ^{230}U/^{226}Th generator has been developed. The focus was hold on obtaining ^{226}Th of high purity in a solution amenable to further labeling. The first column of the proposed generator filled with TEVA Resin held ^{230}U, from which ^{226}Th was eluted with 7 M HCl solution. UTEVA Resin pretreated with nitric acid solution was used as a sorbent for the second column for thorium retention. ^{226}Th was extracted with 0.01-0.05 M citric buffer solution. One cycle of generator milking took 5-7 minutes and produced > 90% of ^{226}Th in 1.5 ml of eluate, pH 4.5-5.0. The proposed two-column ^{230}U/^{226}Th generator was tested over two months including a second loading of ^{230}U additionally accumulated from ^{230}Pa. The ^{230}U impurity in ^{226}Th eluate was less than 0.01% allowing to use it directly in synthesis of radiopharmaceutical compounds.

Keywords: targeted alpha therapy; alpha-emitter; thorium-226; uranium-230; isotope generator; extraction chromatography

1. Introduction

Targetry alpha therapy (TAT) is effective for the treatment of various oncological diseases due to the property of α-particles to release a large amount of energy in a limited area of living tissue (~ 10 cell diameters). One of the promising radionuclides for TAT is ^{230}U ($T_{1/2}$ = 20.2 d) [1]. The decay of ^{230}U generates a chain of short-lived products and it is leads to the emission of five α-particles with a total energy of 33.5 MeV (Figure 1), resulting in effective cell damage [2]. The short-lived daughter alpha emitter ^{226}Th ($T_{1/2}$ = 30.6 min) is also attractive radionuclide for using in TAT [3]. In terms of nuclear properties, the ^{230}U/^{226}Th pair is similar to the well-researched ^{225}Ac/^{213}Bi generator pair [4].

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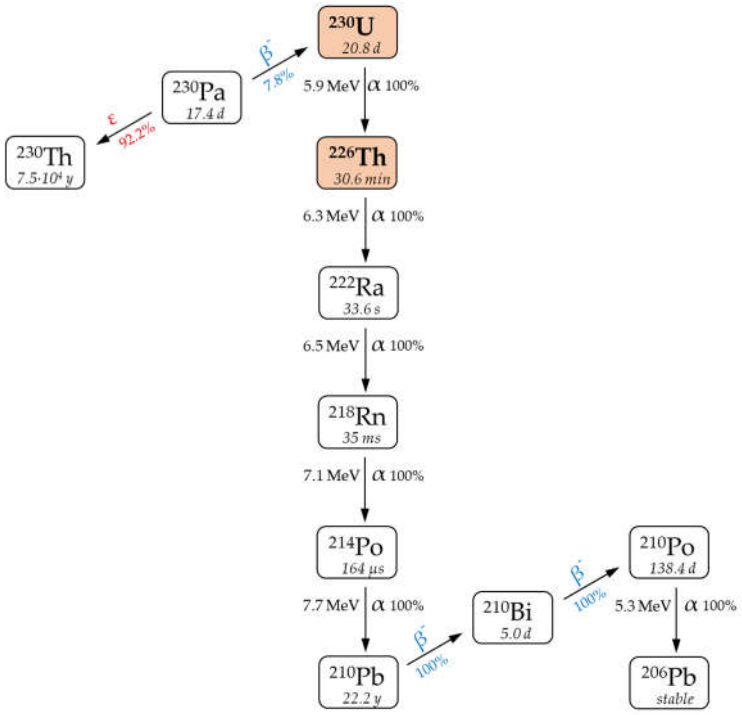

Figure 1. Decay chain of ^{230}Pa [2].

The expected therapeutic efficacy of ^{230}U/^{226}Th has not been demonstrated yet, nevertheless, research is underway to find optimal chelating agents that can stably bind *in vivo* ^{230}U [5] and ^{226}Th [6].

The effective way of ^{230}U and ^{226}Th production is required in order to successfully implement these radionuclides in TAT. ^{230}U can be produced directly via nuclear reactions ^{231}Pa(p,2n)^{230}U [7] and ^{231}Pa(d,3n)^{230}U [8]. The initial ^{231}Pa ($T_{1/2}$ = 3.3·10^4 y) is a decay product of ^{235}U, it must be isolated from aged uranium samples. The raw material is hardly accessible, which makes limitations on this method. Another approach uses reactions of thorium nuclei with accelerated protons and deuterons, leading to the formation of ^{230}Pa precursor decaying into ^{230}U with a branching ratio of 7.8%: ^{232}Th(p,3n)^{230}Pa→^{230}U and ^{232}Th(d,4n)^{230}Pa→^{230}U.

Scientific organizations worldwide are actively developing the production of ^{230}U through irradiation of ^{232}Th with protons [10-13]. This method has proven to be the most effective in terms of product yield compared with the reactions with deuterons [14, 15]. Moreover, the maximum of the ^{232}Th(p,3n)^{230}Pa reaction excitation function is around 20 MeV [9, 15], which enable up-scaled production of ^{230}U on accessible commercial cyclotrons. At higher energy of protons (> 70 MeV) ^{230}U can be obtained as a byproduct in ^{225}Ac production [16, 17] along with ^{223}Ra [18].

^{230}U from proton-irradiated thorium contains a chemically inseparable impurity of long-lived uranium isotopes. This impurity was evaluated in our previous paper [15] as up to 0.02% ^{232}U ($T_{1/2}$ = 68.9 y) and 0.001% ^{233}U ($T_{1/2}$ = 1.6·10^5 y). Long-lived admixture makes the medical use of ^{230}U as a source in a radionuclide generator of ^{226}Th more prospective than a direct application.

^{226}Th is considered to be an alternative to another promising generator-produced short-lived radionuclide ^{213}Bi ($T_{1/2}$ = 45.6 min) [19]. ^{226}Th provides a greater impact on cancer cells compared with ^{213}Bi. A rapid cascade of four α-particles initiated by ^{226}Th decay deposits totally 27.7 MeV, while ^{213}Bi emits only one α-particle with an energy of 8.4 MeV. ^{226}Th-radiopharmaceuticals can be effective for therapy of epithelial or easily accessible tumors [20]. Radioimmunoconjugates Nimotuzumab-p-SCN-Bn-DTPA(DOTA) were synthesized in our previous paper and their specificity towards EGFR overexpressing epidermoid carcinoma A431 cells has been demonstrated [21]. A major advantage of ^{226}Th-TAT compared with ^{230}U-TAT is that there is no problem of uncontrolled redistribution of recoiled daughter nuclide (for ^{230}U such nuclide is ^{226}Th itself), which might cause considerable toxic effects to heathy tissues.

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Due to the relatively short half-life of ^{226}Th, time economy becomes a major requirement throughout the entire process from obtaining ^{226}Th to radiopharmaceutical administration. For this reason, the generator system must ensure the rapid and efficient separation of the accumulated thorium radionuclide. Various methods of liquid-liquid extraction [22, 23], extraction chromatography [24-26] and ion exchange chromatography [27-29] have been developed for the separation of thorium and uranium. For instance, for the selective isolation and determination of U, Th and a number of other radionuclides in water (sample volume up to 1 L), methods based on their sorption with TEVA Resin and UTEVA/TRU Resin sorbents have been recommended [30, 31]. The chromatographic methods are more appropriate for a ^{230}U/^{226}Th generator because they usually provide ^{226}Th in a small volume of eluate containing reduced amount of long-lived impurities.

Effective separation of U(VI) and Th(IV) can be achieved on sorbents displaying anion-exchange properties in strong hydrochloric acid solutions. As it can be seen in Figure 2, U(VI) exhibits high affinity to a strong base anion-exchange resin Dowex 1 (or AG 1) and to an extraction chromatographic resin TEVA at c(HCl) > 6 M, whereas Th(IV) is not retained. The both resins were tested as sorbents for ^{230}U/^{226}Th «direct» generator [21]. TEVA Resin proved to be more preferable, it provided high ^{226}Th yield in less volume of eluate (1-2 mL). Furthermore, the maximal mass distribution ratio D_m of U(VI) adsorbed on TEVA Resin is located around 7 M HCl, whereas the largest adsorption of U(VI) on AG 1 corresponds to HCl concentration greater than 9 M (Figure 2). High acidity of final ^{226}Th eluate is found to be the main disadvantage assuming the extra-time needed to convert the eluate into neutral solution prior to labeling.

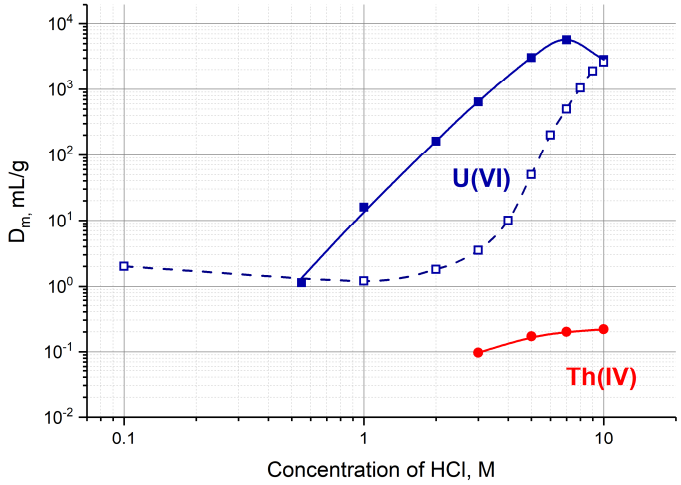

Figure 2. The mass distribution ratios D_m, mg/mL, of U(VI) and Th(IV) upon sorption onto TEVA Resin (solid lines) [30] and Dowex 1x10 (dashed line) [32] as a function of HCl concentration.

Another approach is based on reverse scheme of ^{230}U/^{226}Th generator, i.e. the parent ^{230}U is not fixed on the column filled with sorbent while the daughter ^{226}Th remains adsorbed. An extraction chromatographic DGA Resin containing diglycolamide derivative is reported to be appropriate for obtaining ^{226}Th in citric buffer solution that is amenable to direct labeling with minimal losses of time [12]. However, the ^{226}Th eluate recovered from the reported reverse ^{230}U/^{226}Th generator contains at least 0.2% of ^{230}U [12], which is unacceptable for clinical trials so far.

In the presented article, we investigate different schemes of two-column ^{230}U/^{226}Th generator pursuing two goals: i) obtaining ^{226}Th of high purity in a solution amenable to further labeling; ii) reducing the time of ^{226}Th production. The first column serves for fixing the parent ^{230}U and elution of ^{226}Th. The second column is intended to adsorb ^{226}Th from a strongly acidic solution and then to desorb it with diluted or neutral solution. This concept was early proposed and tested for ^{225}Ac/^{213}Bi generator [33-35]. In the first column, ^{225}Ac formed an extremely strong complex with bis-(2-

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ethylhexyl)methanediphosphonic acid (H2DEH[MDP]) immobilized on a silica support (Ac Resin). Products of ^{225}Ac decay, ^{221}Fr, and ^{213}Bi were eluted with 1 M HCl and concentrated on the second column filled with ion exchanger AG-MP 50 from 0.2 M HCl. Then, ^{213}Bi was eluted from the second column with 0.1 M HI. The high efficiency of this approach makes it suitable for implementation for ^{230}U/^{226}Th pair as well.

2. Materials and Methods

All chemicals were of p.a. (pro analysis) quality or higher, obtained from Merck (Darmstadt, Germany), and used without additional purifications. All experiments were carried out using de-ionized "Milli-Q" water (18 MΩ·cm^{-1}). DGA Resin (N,N,N`,N`tetroctyldiglicolamide as an extracting agent), TEVA Resin (quaternary ammonium salt Aliquate 336 as an extracting agent), TRU Resin (octyl(phenyl)-N,N-di-isobutylcarbomoylmethylphosphine oxide dissolved in tributylphosphate), and UTEVA Resin (dipentil pentylphosphonate as an extracting agent) with 50–150 μm particle size were obtained from Triskem, France.

Citric buffer solutions (10^{-4} – 10^{-1} M, pH 5.0±0.1) were prepared by dissolving the corresponding solid acid sample and adding small portions of 1 M NaOH to obtain a solution with the required pH value.

Measurements of operational pH values were performed with an Orion 2 Star Benchtop pH meter using an Orion 8103SC combination pH electrode. Commercial pH Titrisol buffer concentrates (Merck p.a.) were used to calibrate the setup at room temperature.

Acid-base titration with indicators methyl orange and phenolphthalein was used to determine the acid content in commercial solutions of concentrated HCl and HNO_3, as well as in ^{226}Th containing eluate.

The experiments were carried out at the temperature of 21±2 °C.

2.1. Gamma-ray spectroscopy

The measurement of radionuclide activities was performed by γ-ray spectrometry using a high resolution HP Ge detector (ORTEC GEM15P4-70). Samples were counted at different detector-source distances respecting the level of dead-time less than 10%. The detector efficiency at the used distances were determined with standard calibration sources. Net peak areas in detected photopeaks were evaluated by means of GammaVision32 software.

The characteristic γ-ray emission of ^{226}Th (111.1 keV, 3.29%) and ^{222}Ra (324.3 keV, 2.77%) [2] were used for activity quantification of various generator testing samples.

2.2. Target preparation and irradiation, and ^{230}U isolation

Metallic thorium supplied by Institute for Physics and Power Engineering (IPPE, Russia) was used as target material. Thorium plates of (2.2 × 2.5) cm^2 approximate dimensions with thickness of 1.5-2.0 mm were fabricated and packed in copper and aluminum foil envelopes served for beam monitoring as well. Each package was encapsulated in a graphite shell sealed with high-temperature silicone adhesive. Several targets were irradiated at linear proton accelerator of the Institute for Nuclear Research of the Russian Academy of Sciences (INR RAS, Russia) [36] with initial energy of 120-130 MeV. The beam current and total beam charge were 3-5 μA and 12-18 μA·h, respectively.

The dissolution of irradiated thorium was performed as described previously [17,37] four to five days after the end of bombardment (EOB). The protactinium fraction including ^{230}Pa was recovered from the solution according to the procedure reported [15] and kept in 7 M HCl / 0.1 M HF solution for ^{230}U accumulation during 27-28 days. Traces of Nb (mainly ^{95}Nb) and Ru (^{103,106}Ru) radioisotopes were the impurities of ^{230}Pa/^{230}U.

A chromatographic technique close to the one developed by A.W. Knight [38] was implemented for separation of ^{230}U from ^{230}Pa. The solution with radionuclides was loaded onto a column filled with 2 mL of TEVA Resin. Due to the presence of fluoride ions, Pa(V) together with Ru(IV) were eluted while U(VI) and Nb(V) were retained on the resin. The column was washed with 7 M HCl /

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0.1 M HF solution to remove the rest of Pa(V). The washing was added to the Pa(V) eluate and the combined solution was kept for the next ^{230}U accumulation. Then, having the column been washed with 7 M HCl solution, U(VI) and a part of Nb(V) were desorbed with 0.1 M HCl solution. The desorbate was adjusted to 3 M HCl by adding concentrated hydrochloric acid; and, following the known procedure [12, 39], this solution was passed through a column filled with 1 mL of DGA Resin. The uranium fraction was adsorbed while the ^{95}Nb was washed out of the column. Finally, ^{230}U was eluted with a small amount of 0.1 M HCl solution.

2.3. Generator schemes for producing ^{226}Th

2.3.1. Preparation of a parent ^{230}U column

A plastic column of ~ 5 mm in diameter was filled with 1 mL of TEVA Resin equilibrated with 7 M HCl. The resin was fixed inside by two frits at the bottom and at the top of column. The ^{230}U solution was evaporated and reconstituted in 7 M HCl. The resulting solution was passed through the column followed by washing a solution of 7 M HCl. The uranium was adsorbed under these conditions, the loaded activity of ^{230}U was 300-350 kBq. Approximately a month after the ^{230}U isolation and loading, a second part of ^{230}U was accumulated, separated from ^{230}Pa as described above and added to the column.

The prepared column served as a parent one, it could work as a one-column generator providing ^{226}Th elution in 7 M HCl. A typical differential curve of ^{226}Th elution is shown in Figure A1. Furthermore, the parent column was a part of two-column generator schemes.

2.3.2. Two-column ^{230}U/^{226}Th generator scheme and ^{226}Th elution cycle

A general scheme of ^{230}U/^{226}Th generator comprised two columns connected in series via a three-valve cock as shown in Figure 3. The parent TEVA column containing ^{230}U was the first column and a column filled with TRU, UTEVA or DGA Resin served as a second one that could be changeable when necessary. A milking procedure included two common steps: 1) transfer ^{226}Th with 7 M HCl solution from the parent column to the second one (Figure 3a); 2) ^{226}Th elution with diluted HCl or citric buffer solution (Figure 3c). Eluates were collected for measurement of ^{226}Th and ^{230}U activity and eluate acidity. Flow rates of solutions passing through the columns were kept and controlled with a peristaltic pump. The values of flow rate were 1 and 0.6 ml/min for Step 1 and 2, respectively.

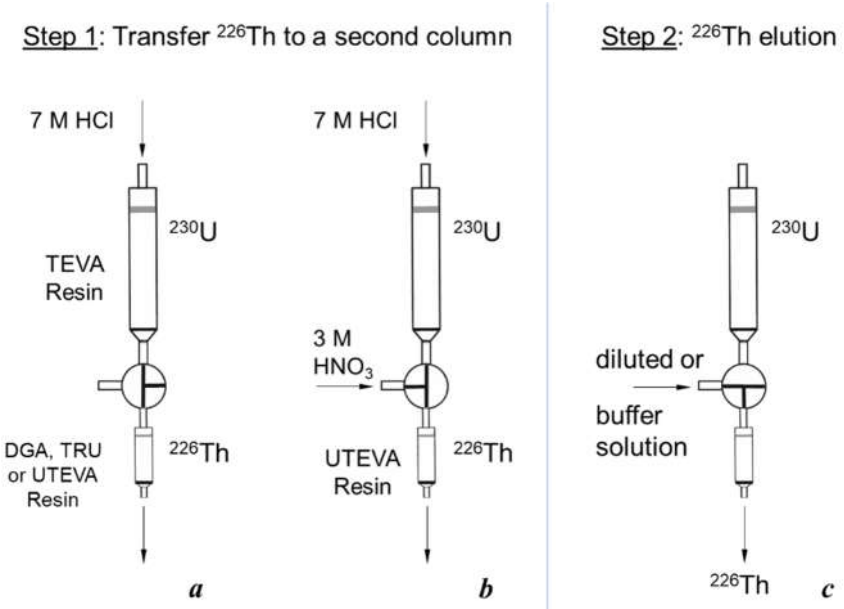

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Figure 3. Scheme of ^{226}Th production by a two-column ^{230}U/^{226}Th generator: a, b – two modifications of transferring ^{226}Th from the parent ^{230}U column to a second one; c – elution of ^{226}Th from the second column.

The TRU or DGA Resin was loaded into a plastic column of ~ 3 mm in diameter, the height of resin bed was 11-12 mm (resin volume ~ 0.1 mL). A diameter of column for UTEVA Resin was ~ 5 mm, the height of UTEVA bed was 55-58 mm (~ 1 mL). Each column was equipped with a bottom and top frits.

For some experiments with the UTEVA column, we modified Step 1 as it is shown in Figure 3b. A flow of 7 M HCl solution (0.5 mL/min) after passing the parent ^{230}U column was mixed with a flow of 3 M HNO_3 solution (0.5 mL/min) resulting in the 1 ml/min flow of 3.5 M HCl / 1.5 M HNO_3 solution at the entrance of UTEVA Resin column.

2.4. ^{230}U measurements

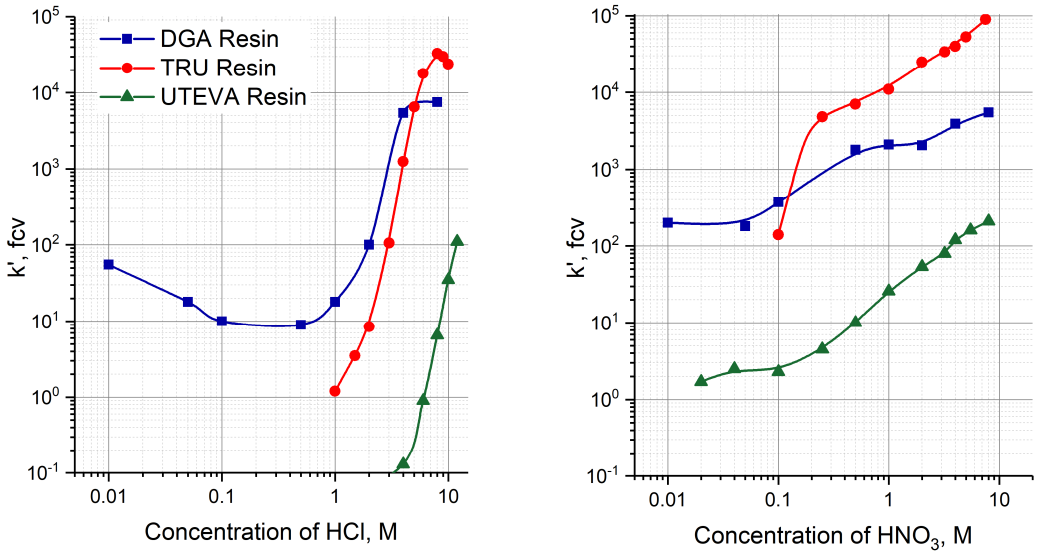
The content of ^{230}U in eluates was usually measured overnight for complete ^{226}Th decay. ^{230}U was assayed by γ-ray spectroscopy via the daughter radionuclides ^{226}Th and ^{226}Ra.

Distribution of ^{230}U along the TEVA Resin column was monitored by scanning through a 4 mm wide slit between the lead blocks. The measurements were performed after ^{230}U loading onto the TEVA Resin column and regularly after milking.

3. Results and Discussion

A two-column ^{230}U/^{226}Th generator was proposed and investigated for fast ^{226}Th production in diluted or neutral citric solution. The parent ^{230}U was adsorbed onto the first column filled with TEVA Resin (Triskem Int.). On reaching the transient equilibrium, the daughter ^{226}Th was separated and eluted with strong HCl solution. The role of second column was to reduce quickly the acidity of ^{226}Th solution, i.e. a sorbent for second column was expected to retain ^{226}Th from the strong HCl solution and to desorb it into a diluted solution. Three extraction chromatographic resins eligible for this purpose were considered: TRU Resin, UTEVA Resin and DGA Resin (all Triskem Int.). According to the reported data [40-42] obtained in static conditions and shown in Figure 4a, the resins can be arranged in a row with respect to Th(IV) retention from < 5 M HCl solution:

DGA Resin > TRU Resin > UTEVA Resin



(**a**) (**b**)

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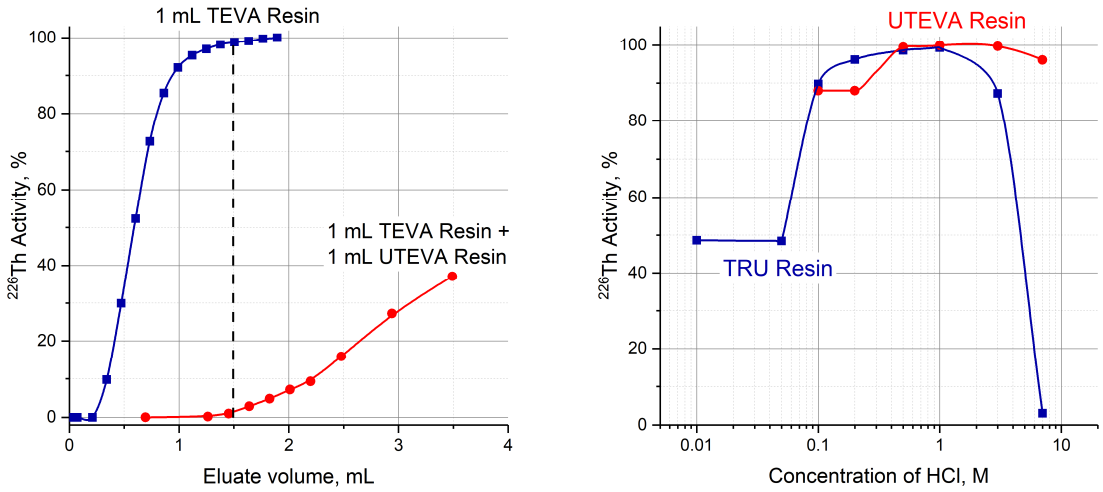
Figure 4. Capacity factors k', fcv, of Th(IV) upon sorption onto DGA, TRU and UTEVA Resins as a function of HCl (**a**) and HNO_3 (**b**) concentration [40-42].

In order to evaluate the feasibility of two-column $^{230}U/^{226}Th$ generator, the column experiments on ^{226}Th sorption from 7 M HCl and its desorption with diluted HCl solutions were carried out.

3.1. Elution of ^{226}Th from the second column with HCl solutions

First, the transfer ^{226}Th to a second column was investigated (Figure 3a, Step 1). ^{226}Th was easily stripped off the parent column with 7 M HCl solution, the concentration corresponding to the maximum of U(VI) sorption on TEVA Resin (Figure 2). The integral ^{226}Th elution curve (blue line in Figure 5a) indicates that the solution volume of 1.5 mL was sufficient to wash out ≥ 99% of ^{226}Th. DGA Resin and TRU Resin displays high adsorption of ^{226}Th from 7 M HCl solution (Figure 4a), the values of k' Th(IV) attain 10^4. When the second column was filled with 0.1 mL of these resins and connected directly to the exit of parent column, ^{226}Th was completely adsorbed onto the second column. In contrast, the values of k' Th(IV) on UTEVA Resin are below 10 under the same conditions. Therefore, the quantity of UTEVA Resin in the second column was increased up to 1 mL to ensure a tolerable breakthrough of ^{226}Th less than 3% (red line in Figure 5a).



(**a**) (**b**)

Figure 5. (**a**) Estimation of UTEVA resin's optimal quantity; (**b**) Efficiency of ^{226}Th desorption from TRU and UTEVA columns as a function of HCl concentration.

The transferred ^{226}Th was eluted with different HCl solutions as it is shown in Figure 3c (Step 2). The DGA Resin exhibits the greatest retention of ^{226}Th among the studied resins from diluted hydrochloric solutions (Figure 4a). Our results of column experiments were in a good agreement with the k' data. The elution of ^{226}Th with 0.3 M HCl solution, which is the most favorable for ^{226}Th desorption, resulted in 40% of ^{226}Th yield in 6 mL of eluate. For other HCl concentrations, the ^{226}Th yield was even lower.

The efficiency of ^{226}Th desorption from the second columns filled with TRU and UTEVA Resins versus the concentration of hydrochloric solution is presented in Figure 5b. The optimal range of ^{226}Th desorption was 0.4-1 M HCl for TRU Resin and 0.5-2 M HCl for UTEVA Resin. Typical ^{226}Th elution curves (Figure 6) display that ^{226}Th was completely eluted in ~ 1 mL of eluate. It is interesting to note that the width of ^{226}Th chromatographic peaks from the TRU Resin and UTEVA Resin columns is almost the same, although the bed volume of UTEVA Resin is 10 times larger than that of TRU Resin.

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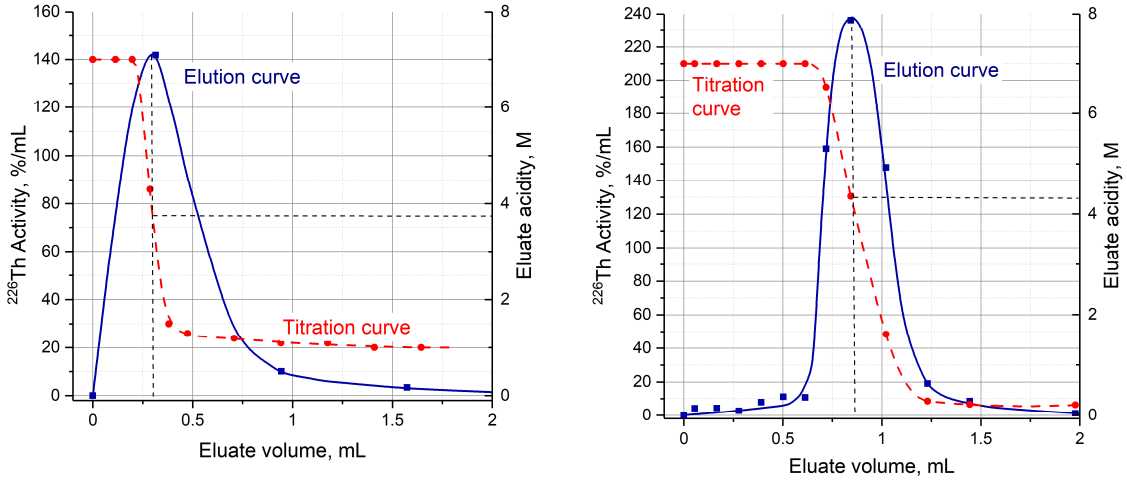

Figure 6. Typical ^{226}Th elution and titration curves. (**a**) Elution of ^{226}Th from TRU Resin with 1 M HCl; (**b**) Elution of ^{226}Th from UTEVA Resin with 0.2 M HCl.

Despite the fact that the HCl concentration of the solution entering the second column was relatively low, the eluate acidity from both TRU Resin and UTEVA Resin columns was 3-4 M [H^+]. The detailed titration curves of eluate collected by portions (Figure 6) demonstrate that ^{226}Th is eluted on the HCl concentration drastic gradient when one solution is replaced by another. Maximum of ^{226}Th chromatographic peak corresponds to H^+ concentration around 4 M. Thus, use of dilute HCl solutions allowed us to decrease eluate acidity by only two times.

3.2. Elution of ^{226}Th from the second column with citric buffer solutions

After transferring from the parent column to a second one containing TRU, UTEVA or DGA Resin, ^{226}Th can be desorbed with a neutral citric buffer solution. The efficiency of ^{226}Th desorption was studied as a function of H_3Cit (pH 5.0) concentration in the range of 10^{-4} M – 10^{-1} M. The dependencies plotted on the graph (Figure 7) are arranged in the order reflecting the above-mentioned sequence of resins' affinity for thorium (IV). For all the resins, 0.1 M citric buffer solution wholly recovered ^{226}Th.

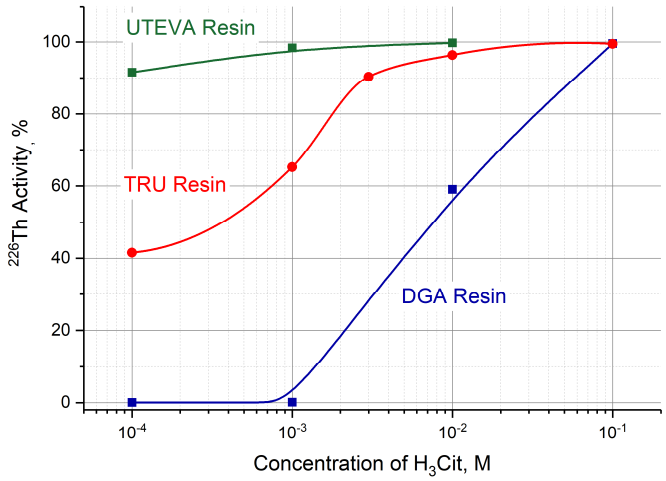

Figure 7. Efficiency of ^{226}Th desorption from DGA, TRU and UTEVA Resin columns as a function of citric acid concentration.

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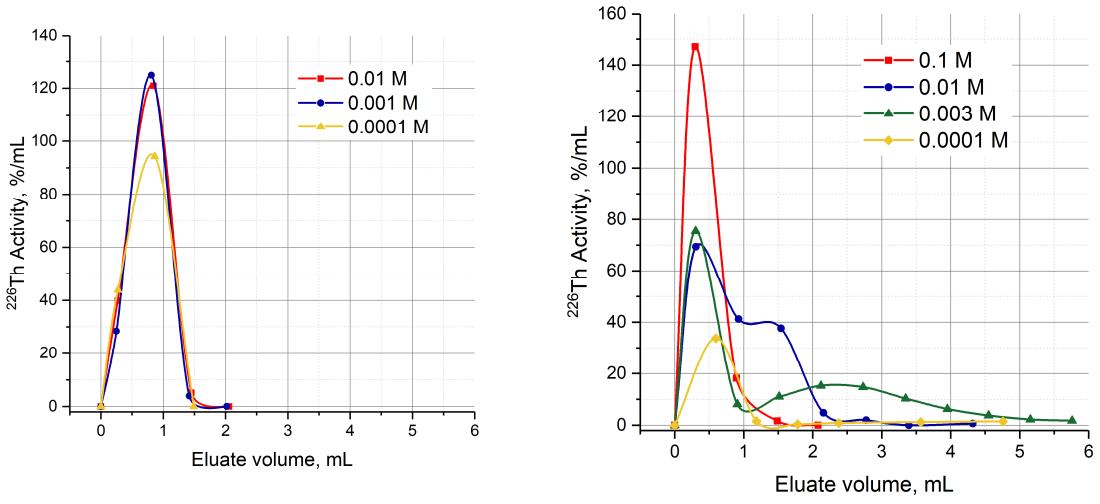
Typical curves of ^{226}Th elution from the UTEVA Resin and DGA Resin columns consisted of the only chromatographic peak within the given range of citric acid concentration (Figure 8a). Similar to the ^{226}Th elution with dilute hydrochloric solutions, the peak maximum followed the acidity gradient. Otherwise, two chromatographic peaks were observed when ^{226}Th was eluted from the TRU column with citric buffer solutions (Figure 8b). The first peak was in the same way related to HCl concentration gradient. The position of maximum V_{max} of second peak, as well as the capacity factor k' defined as $k' = \frac{V_{max} - V_c}{V_c}$ [40] (V_c is free volume of sorbent in a column), depended on the citric acid concentration.



(**a**) (**b**)

Figure 8. Curves of ^{226}Th elution with citric buffer solutions of different concentration. (**a**) Elution from UTEVA Resin; (**b**) Elution from TRU Resin.

According to the literature data, various complexes of Th(IV) are coexisting in citric acid media depending on pH values and salinity [43, 44]. At pH 5-6 the predominant species are $ThCit_3^{5-}$ and $ThCit_2(OH)_2^{4-}$ [43] with cumulative formation constants (logβ) of 28 and 15, respectively. In more acidic solution (pH<1) the speciation shifts towards cation forms of Th(IV), e.g. Th^{4+} and $ThCit^{+}$ (logβ = 14). It is evident that all these species may exhibit different affinities to the resins and the detailed analysis is complicated. However, in the case of TRU Resin the dependence of k' Th(IV) on citric acid concentration may be expressed by a simple correlation helpful for practice use:

$$k' = \frac{k'_0}{1 + a \cdot [\mathrm{H_3Cit}]}$$

where k'_0 and a are empiric constants.

Satisfactory values of ^{226}Th yield (> 90%) in small amount of eluate (1-1.5 mL) were obtained for all the studied resins, the eluate characteristics are listed in Table 1.

Table 1. Acidity of ^{226}Th eluate from the second column of ^{230}U/^{226}Th generator (eluate volume 1 mL).

Resin	Concentration of citrate buffer solution (eluent), M	Eluate acidity, M
UTEVA	10^{-3} – 10^{-1}	1.9 – 2.1
TRU	10^{-1}	1.6 – 1.7
DGA	10^{-1}	1.6 – 1.7

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It was found that eluate acidity remained relatively excessive for immediate synthesis of labeled compounds. In order to keep ^{226}Th on the second column during the substitution of acidic medium with neutral one, the influence of nitrate ions was studied.

3.3. Stabilization of ^{226}Th on the second column before elution

The ability of Th(IV) to form stable anionic complexes with nitrate ions is widely used to separate it from other elements. Comparison of *k'* values for DGA, TRU and UTEVA resin reveals higher sorption of Th(IV) from nitric solutions (Figure 4b) than from hydrochloric ones (Figure 4a), especially for the acidity below 1 M. Taking this fact into account, we modified the procedure of ^{226}Th production from the two-column ^{230}U/^{226}Th generator.

The initial part of modification consisted in the pre-treatment of second column. Before transferring ^{226}Th from the parent column (Step 1), 10 mL of HNO_3 solution of a certain concentration was passed through the second column with the flowrate of 1 mL/min. For the DGA and TRU Resins, HNO_3 concentration was 0.1 M, which corresponds to moderate values of capacity factor 150 < *k'* Th(IV) < 350 (Figure 4b). In the case of UTEVA Resin, the values of k' Th(IV) for dilute nitric acid solutions are small; they grow with increasing acid concentration and reach values around 100 in the region of 3-4 M HNO_3.

Starting from these data obtained in static conditions, the UTEVA column was pre-treated with a HNO_3 solution of various concentrations and ^{226}Th losses during Step 1 were studied. The results presented in Figure 9 display that the ^{226}Th losses when transferring from the TEVA column to UTEVA one with 7 M HCl solution noticeably diminished along with increasing the concentration of HNO_3 used for UTEVA pre-treatment. For 3 M HNO_3 solution, the breakthrough of ^{226}Th began after passing not less than 2.5 mL of 7 M HCl.

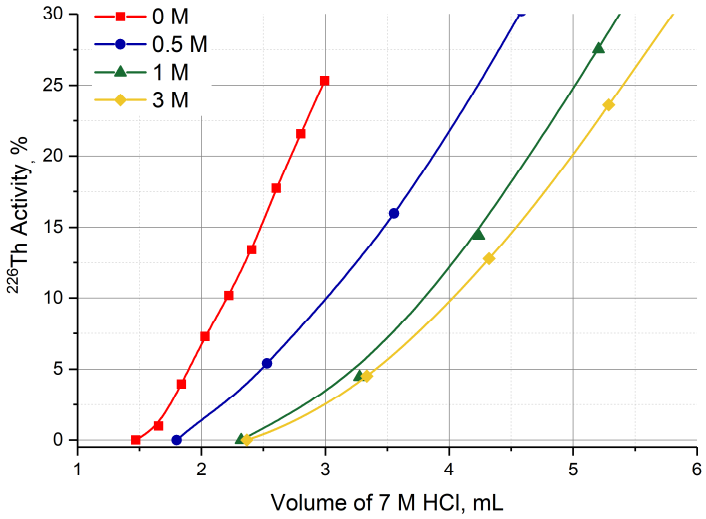

Figure 9. Estimation of HNO_3 concentration for the pre-treatment of UTEVA Resin column.

In addition, a variation of Step 1 was tested (Figure 3b) that provided the flow of 7 M HCl solution carrying ^{226}Th to be equally mixed with the flow of 3 M HNO_3 solution at the entrance of the UTEVA column ("two flows" variation). As a result, ^{226}Th breakthrough was not observed even after 20 mL of 7 M HCl (> 20 bed volumes).

The other part of the second column modification was carried out after Step 1 and involved substituting the 7 M HCl medium with dilute or neutral one. Solutions of 0.1 M HNO_3, 0.15 M NaCl and 0.15 M $NaNO_3$ were studied. Full change of medium in the DGA column took place after passing 1.0-1.2 mL of each solution, and ^{226}Th losses did not exceed 3%. In the case of TRU column, ^{226}Th was partially washed out on the HCl concentration gradient (similar to Figure 6b) regardless of the

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substituting solution, its losses ranged from 10% to 40% and were poorly reproducible. The UTEVA Resin retained well ^{226}Th when the solutions of 0.1 M HNO_3 and 0.15 M $NaNO_3$ were passed through the second column, whereas the 0.15 M NaCl solution washed out up to 28% of ^{226}Th (Figure 10).

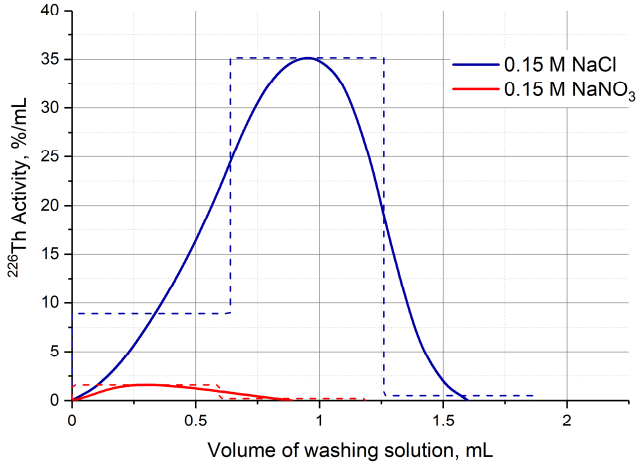


Figure 10. ^{226}Th losses during preliminary diminishing of acidity in the UTEVA Resin column with neutral salt solutions.

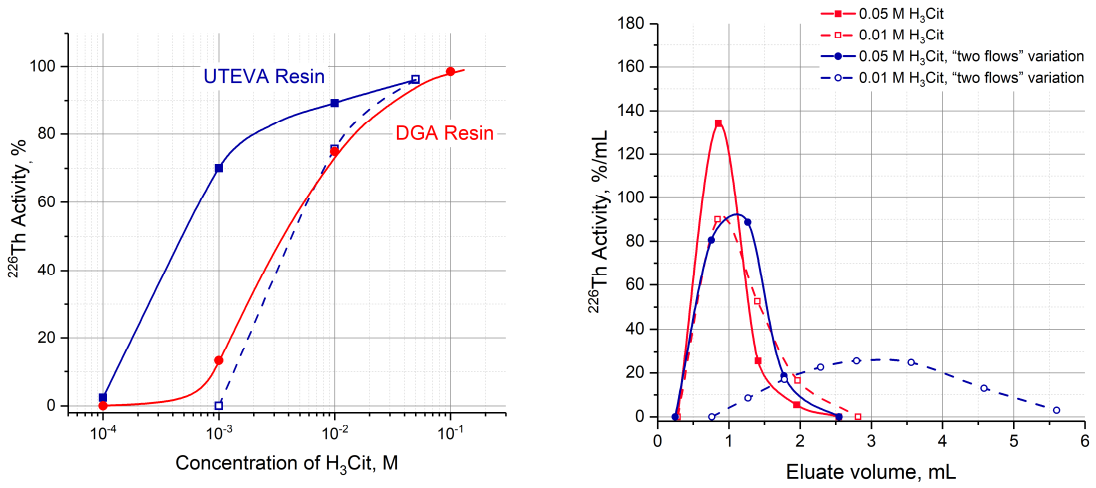
Having replaced the strongly acidic medium in the second column, ^{226}Th was washed out with a citric buffer solution (pH 5.0) of various concentrations (Figure 11a). The results obtained for the UTEVA column:

i) without the pre-treatment and medium substitution (green line in Figure 7);

ii) with the pre-treatment and substitution of 7 M HCl with 0.15 M $NaNO_3$ solution (blue solid line in Figure 11a);

iii) with the pre-treatment, "two flows" variation and the substitution (blue dashed line in Figure 11a)

allow us to suggest that increasing contact time of the UTEVA Resin and nitric solution led to more and more difficult ^{226}Th recovery. The relative positions of ^{226}Th elution curves (Figure 11b) are also in line with the suggestion.



(**a**) (**b**)

Figure 11. (**a**) Different modes of ^{226}Th recovery with citric buffer solutions: from UTEVA Resin column pre-treated with 3 M HNO_3 (blue solid curve), ^{226}Th elution with "two flows" variation (blue

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dashed curve), and from DGA Resin columns pretreated with 3 M HNO_3 (red solid curve); (**b**) Curves of ^{226}Th elution depending on the variations of ^{226}Th recovery from UTEVA columns.

Moreover, the influence of the contact time was evaluated in parallel experiments that included the 3 M HNO_3 pre-treatment of UTEVA column, the HCl substitution with 0.15 M $NaNO_3$ solution and the ^{226}Th elution with a 10^{-3} M citric buffer solution. As the pre-treatment duration increased from 10 minutes to 10 hours, the efficiency of ^{226}Th production decreased from 70% (see in Figure 11a) to zero. The behavior of UTEVA resin may be explained by the fact that the *k'* Th(IV) dependence in nitric solutions is higher than that in hydrochloric solutions over the entire concentration range (Figure 4).

Two most effective procedures for obtaining ^{226}Th from the two-column $^{230}U/^{226}Th$ generator are presented in Table 2.

Table 2. Effective procedures of ^{226}Th extraction from the two-column $^{230}U/^{226}Th$ generator (> 90% in 1.5 ml of eluate, pH 4.5-5.0).

Step	Solution	
	for DGA column	for UTEVA column
Second column pretreatment	0.1 M HNO_3	3 M HNO_3
Transfer ^{226}Th to a second column	7 M HCl	
Acid substitution in a second column	0.15 M NaCl	0.15 M $NaNO_3$
^{226}Th elution	0.1 M H_3Cit, pH 5.0	0.05 M H_3Cit, pH 5.0

The overall time of ^{226}Th eluate production was within 5-7 minutes. The time expenditure was slightly shorter when the DGA column was used, because its size was 10 times smaller than that of the UTEVA column. Meanwhile, the use of UTEVA Resin for the second column resulted in the high yield of ^{226}Th in a less concentrated solution (0.01-0.05 M H_3Cit, pH 4.5-5.0).

3.4. Long-term performance of two-column $^{230}U/^{226}Th$ generator

Following the developed procedure, the solution of 7 M HCl was only passed through the parent column containing ^{230}U adsorbed on TEVA Resin. The distribution of ^{230}U along with the length of parent column was monitored throughout the generator lifetime (Figure A2). Usually, two cycles of ^{226}Th production per weekday were performed over two months. The total volume of 7 M HCl solution passed through the parent column was about 200 mL including accessory operations and a loading of second portion of ^{230}U. According to the calculation illustrated by Figure A3, one third of the initial amount of ^{230}U was additionally accumulated from ^{230}Pa in 27-28 days after the first separation and then loaded onto the parent column.

Due to the two-column scheme of ^{226}Th production, the proposed generator provided deep purification ^{226}Th from ^{230}U. It was found that the ^{230}U impurity in the ^{226}Th eluates did not exceed 0.01%, which was at least one order of magnitude better in comparison with the literature data reported [12].

4. Conclusions

We have proposed and tested the proof-of-concept of a two-column $^{230}U/^{226}Th$ generator for rapid producing ^{226}Th amenable to further labeling. The first ^{230}U column with TEVA Resin furnished ^{226}Th in 7 M HCl solution. The second column retained ^{226}Th from the strongly acidic solution and then released it with a diluted hydrochloric or neutral citric buffer solution. Analysis based on the dependence of capacity factor *k'* Th(IV) on the concentration of hydrochloric and nitric acid allowed us to consider DGA, TRU and UTEVA Resins as promising sorbents for the second column.

High yields (> 97%) of ^{226}Th elution from TRU and UTEVA Resins with a small volume (~ 1 mL) of diluted HCl solutions were obtained. However, the resulting acidity of eluate was 3-4 M $[H^+]$ regardless of the solution concentration entering the second column. The titration analysis displayed that ^{226}Th was eluted on the HCl concentration gradient when one solution was replaced by another.

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Elution of ^{226}Th transferred to the second column containing DGA, TRU or UTEVA Resin was studied with citric buffer solutions (pH 5.0) in two modes. Direct ^{226}Th desorption was also influenced by the acidity gradient. While ^{226}Th was stripped off the UTEVA and DGA columns in one chromatographic peak, a typical curve of ^{226}Th elution from the TRU column consisted of two chromatographic peaks within the studied range of citric acid concentration. The first peak followed the HCl concentration gradient, and the second one may be attributed to ^{226}Th complexation with citrate ions in the course of TRU column elution. The satisfactory yields were achieved by ^{226}Th elution from the second column filled with any of the studied resins. For TRU and DGA Resins, 1-1.5 mL of 0.1 M H_3Cit (pH 5.0) solution extracted more than 90% of ^{226}Th, while the UTEVA Resin column demonstrated similar effectiveness with less concentrated citric buffer solutions (down to 10^{-3} M H_3Cit). The acidity of citric eluates was about two times lower than the diluted HCl solution's eluates but still relatively high for immediate labeling.

The neutral citric-buffered ^{226}Th eluates were obtained when nitrate ions were introduced. The second column was initially put in contact with a nitric acid solution. Then, after ^{226}Th transfer from the parent column, the acidic medium of second column was substituted with neutral one keeping ^{226}Th immobile. The solution of 0.15 M NaCl and of 0.15 M $NaNO_3$ were used for the DGA and UTEVA column, respectively. Finally, ^{226}Th was extracted with citric buffer solution: 0.1 M H_3Cit from the DGA column and 0.01-0.05 M H_3Cit from the UTEVA one. Thus, one cycle of generator milking took 5-7 minutes and produced > 90% of ^{226}Th in 1.5 ml of eluate, pH 4.5-5.0.

The proposed two-column ^{230}U/^{226}Th generator was tested over two months including a second loading of ^{230}U additionally accumulated from ^{230}Pa. The ^{230}U impurity in ^{226}Th eluate was less than 0.01% allowing to use it directly in synthesis of radiopharmaceutical compounds.

Author Contributions: Conceptualization: S. Ermolaev; methodology: S. Ermolaev and A. Skasyrskaya; validation: S. Ermolaev, A. Skasyrskaya and A. Vasiliev; formal analysis: S. Ermolaev and A. Skasyrskaya; investigation: S. Ermolaev, A. Skasyrskaya and A. Vasiliev; writing—original draft preparation: S. Ermolaev and A. Vasiliev; writing—review and editing: S. Ermolaev and A. Vasiliev; visualization: S. Ermolaev and A. Vasiliev; supervision: S. Ermolaev; funding acquisition: S. Ermolaev. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

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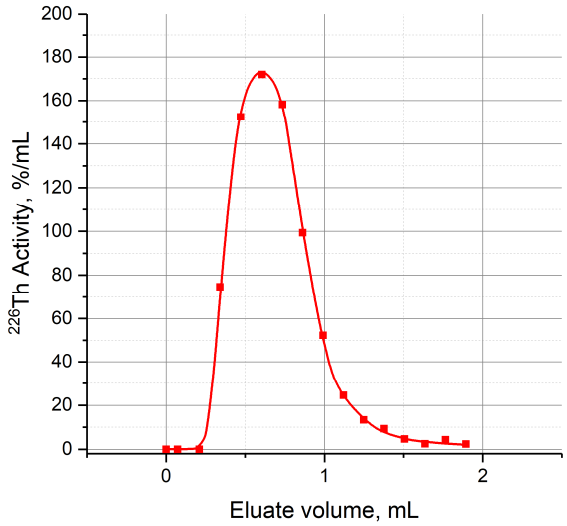

Figure A1. Curve of ^{226}Th elution from TEVA Resin column.

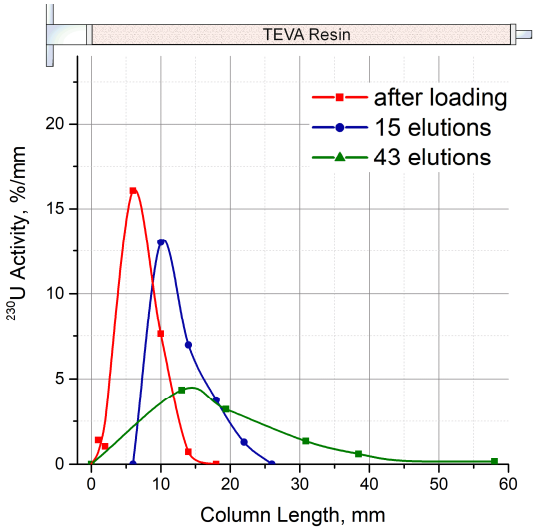

Figure A2. Distribution of activity along with the length of TEVA Resin column (0.4 mL) in the ^{230}U/^{226}Th generator depending on the number of elutions.

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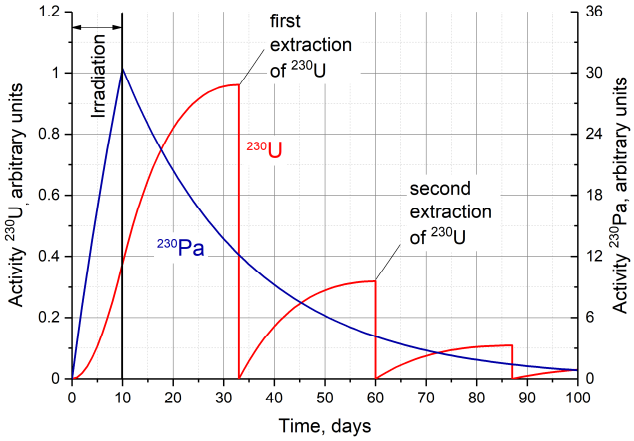

Figure A3. Activity of ^{230}Pa produced by a typical irradiation of thorium target [15] following by its isolation and periodic extraction of ^{230}U. .

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