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Original Article

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Real-time identification of the separated lanthanides by ion-exchange chromatography for no-carrier-added Ho-166 production

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ABSTRACT

No-carrier-added holmium-166 (n.c.a ^{166}Ho) separation is performed based on the results of separation conditions using stable isotopes dysprosium (Dy) and holmium (Ho) to minimize radioactive waste from separation optimization procedures. Successful separation of two adjacent lanthanides was achieved by cation-exchange chromatography using a sulfonated resin in the H+ form (BP-800) and α-hydroxyisobutyric acid (α-HIBA) as eluent. For the identification process after separation of stable isotopes, the use of chromogenic reagents alternatively enables on-line detection because the lanthanides hardly absorb light in the UV-vis region or exhibit radioactivity. Four different chromogenic reagents were pre-tested to evaluate suitable coloring reagents, of which 4-(2-Pyridylazo)resorcinol is the most recommendable considering the sensitivity and specificity for lanthanides. Lanthanide radioisotopes (RI) were monitored for separation with an RI detector using a lab-made separation LC system. Under the proper separation conditions, the n.c.a ^{166}Ho was effectively obtained from a large amount of 100 mg dysprosium target within 2 hrs.

Key Word: Lanthanide separation, Holmium, No-carrier added, On-line detection, Cation-exchange chromatography

Introduction

Lanthanides have received significant interest as an essential element for industrial applications, such as fingerprint powders, light-emitting devices, petroleum refining catalysis, permanent magnets, and telecommunication, due to their unique optical, magnetic, and electronic properties (1-3). In particular, bioscientific applications have been gaining attention in fields of medical analysis and imaging (4). Certain lanthanides with radioactivities have been used as theranostic radionuclides in nuclear medicine because they have an appropriate half-life and release suitable beta-energy for cancer treatment (5). Radiolanthanides emitting β^- particles, such as ^{161}Tb, ^{166}Ho, ^{170}Tm, and ^{177}Lu, have been widely studied and used in radiotherapeutic applications. Representatively, ^{166}Ho ($t_{½}$ = 26.6 h) has interesting physical decay properties for internal radiation therapy, such as high-energy β-radiation ($E_{\beta 1}$ = 1,855 keV

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(51%), $E_{\beta 2}$ = 1,776 keV (48%)), which has a maximum soft tissue penetration range of 8.7 mm, and low-energy γ-rays (80.5 keV, 6%), which is suitable for imaging (6). Exploiting these advantages, KAERI (Korea Atomic Energy Research Institute) developed ^{166}Ho-chitosan complex as a radiotherapeutic agent for cancer therapy (7). However, since ^{166}Ho was produced through direct neutron irradiation (n, γ) of a ^{165}Ho target, the isotopes produce a carrier added state and contain long-lived ^{166m}Ho ($t_{½}$ = 1,200 y) impurities. ^{166}Ho and these by-products result in low specific radioactivity and also unnecessary exposure to patients.

No-carrier-added (n.c.a) radiolanthanides are a key form with high specific radioactivity and high radionuclidic purity. Unlike the direct method, an indirect production route can produce nca radiolanthanides. This approach entails neutron capture of an enriched target and subsequent decay followed by radiochemical separation and purification from the target materials. This production route can provide a free form without the long-lived radionuclidic impurities. ^{166}Ho can be obtained in carrier-free via double neutron capture (2n, γ) of a ^{164}Dy target and subseqeunt β- decay of produced ^{166}Dy ($t_{½}$ = 81.6 h), as follows: ^{164}Dy(n,γ) ^{165}Dy(n,γ) ^{166}Dy → ^{166}Ho (8). The important aspect is that a trace amount of the desired elements should be extracted from macroscopic amounts of the targets. Among the suggested approaches, the methods using ion-exchange chromatography have been assessed to be the most effective in terms of separation. These approaches are based on slight differences in the stability constant between both adjacent lanthanides and a complexing agent, which affects the elution order of the metals (5). In general, the separation setup consists of an HPLC system equipped with a cation-exchange column and α-hydroxyisobutyric acid (α-HIBA) as an eluent and a complexing agent (9).

To determine the optimal conditions for the separation of radiolanthanides, an alternative separation tests using stable isotopes has been proposed. After stable isotope separation was performed, the identification techniques that were mainly used are X-ray fluorescence (XRF), inductively coupled plasma-mass spectrometry (ICP-MS), and atomic absorption spectroscopy (AAS). These techniques can provide accurate analytical results for lanthanides, but require a complicated process and long analysis times. In addition, it is difficult to ascertain the exact elution time because the sample is taken at fixed time intervals (e.g. every 5 or 10 min), which can lead to elution-time errors. To overcome these shortcomings, a post-column derivatization agent was used to immediately detect the separated lanthanide. Several researchers have studied the on-line determination of lanthanides in chromatographic separation fields (10-12). R. M. Cassidy performed a rare earth metal analysis on rocks using Arsenazo III as a post column reagent and M. R Buchmeiser *et al.* analyzed rare earth metals by comparing two reagents, PAR (4-(2-pyridylazo)resorcinol) and Arsenazo III. Veronika Mocko *et al.* attempted to detect the separation of Ho-163 from the Dy target by post-column derivatization with PAR followed by spectrophotometric detection. The basic principle and compositions of the post-column reaction are as follows. Post-column reactions are based on spectrophotometric measurement for the chemical reaction between the appropriate chromogenic complexing agent, the so-called post-column reagent, and the metal after the sample is eluted from the column. This system has been used to improve the sensitivity and specificity for the on-line determination of metals (13).

This study focuses on simple and alternative process for separating adjacent stable isotopes by immediate detection with a post-column reagent to determine the appropriate separation conditions. This process reduces the large amounts of radioactive waste that can be gener-

ated during the experimental evaluation of the radioisotope itself. The on-line detection system provides a variety of information such as column reuse and reproducibility within a short time. Preliminary tests with four different chromogenic reagents showed that PAR is the most suitable post-column reagent due to its high sensitivity and specificity with lanthanides. Furthermore, the separation conditions found in this system were applied to medical radiolanthanide preparation.

Materials and Methods

Materials and Instruments

All reagents were used without any further purification and all solutions were prepared with deionized water obtained from Mirae ST Co., Ltd. Lanthanide chlorides, $DyCl_3 \cdot 6H_2O$ and $HoCl_3 \cdot 6H_2O$, were obtained from Sigma-Aldrich. α-HIBA and chromogenic reagents, including PAR (4-(2-pyridylazo)resorcinol), Arsenazo III (2,7-bis(o-arsenophenyl)azo-1,8-dihydroxynaphtalene-3,6-disulfonic acid), Xylenol orange (3,3′-bis [N,N-bis(carboxymethyl)aminomethy]-*o*-cresolsulfonphthalein disodium salt), and Cupferron (*N*-nitroso-N-phenylhydroxylamine ammonium salt), were purchased from TCI. Hydrochloric acid, ammonium hydroxide, and glacial acetic acid were purchased from Daejung Chemicals and Merck, respectively. Cation exchange resin, BP-800 (H^+ form, 9 μm), was purchased from Benson Polymeric Inc. A syringe pump, a Fusion 200, was purchased from Chemyx Inc. A UV-Vis spectrophotometer (Agilent 8453) was used for lanthanide complex detection. 0.1 M α-HIBA solution was prepared by adding 10.41 g of α-HIBA to a flask and adjusting the pH to 4.2 with water and ammonia to prepare a 1 L solution. This eluent was filtered through a 0.2 μm filter and stored at 4 ℃

Preliminary study of the post-column reaction

Lanthanide solutions (200 ppm) for the pretest were prepared by dissolving the corresponding amount of chloride in distilled water, and working solutions were obtained by dilution with distilled water. The aqueous stock solution of post-column derivatization reagents was prepared as follows: 0.1 mM PAR solution (λmax 415 nm, yellow color) in 1.5 M ammonium hydroxide and 1 M acetic acid (pH 9.8) (11); 0.1 mM Arsenazo III solution (λmax 540 nm, red color) in 1 M acetic acid (pH 2.4) (14); 0.1 mM Xylenol orange solution (λmax 435 nm, intense yellow color) in 1 M acetic acid and 6 M NaOH (pH 5.8) (15); 0.1 mM Cupferron solution (λmax 268 nm, colorless) (16).

To perform simple preliminary work on the post-column reagents, 0.7 ml of each reagent was mixed with 1.0 ml of 0.1 M α-HIBA and 0.3 ml of 100 ppm Dy^{3+} solution. Figure 1 shows the molecular structure of each reagent and the characteristic color change before and after the addition of lanthanide. PAR, Arsenazo III, and Xylenol orange change from pale yellow to red, red purple to bluish green, and orange to pink, respectively, under appropriate discoloring pH range. Under the same conditions where only the lanthanide is changed to Ho^{3+}, color changes show the same patterns as in the previous experiment with Dy^{3+}.

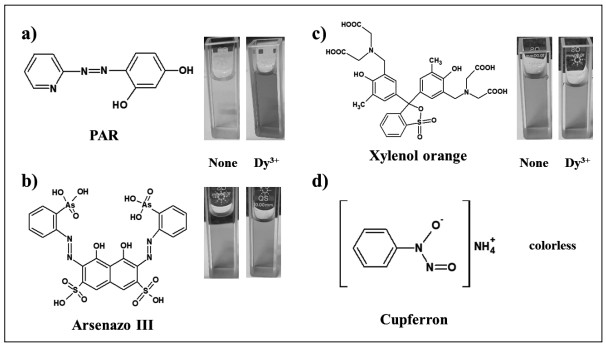

Figure 1. The molecular structures of a) PAR, b) Arsenazo III, c) Xylenol orange and d) Cupferron, and their chromogenic responses to Dy^{3+} ion. The solution was prepared by mixing 0.1 M α-HIBA (1.0 ml), each reagent (0.7 ml) and distilled water or Dy^{3+} solution (0.3 ml).

LC system with the post-column detector

The LC system is composed of a chromatographic pump for separation, a syringe pump for transferring chromogenic reagents, and a UV-vis spectrophotometer containing a flow cell for lanthanide detection (17). The 6-port Rheodyne valve equipped with a 500 μl sample loop is connected with an Ultra High Pressure Constant Pump (A P-UHP-CP model) of Chrom Tech Inc. The LC column was prepared by using an Eco Plus glass column (YMC, i.d. 10 mm) as follows:

Column A. A 1.0x5.5 cm column was packed with 5 g of cation exchange resin.

Column B. A 1.0x16 cm column was packed with 14 g of cation exchange resin.

After column packing was completed, the column conditioning was carried out for 5 hrs by eluting 2 ml/min of 0.1 M α-HIBA. The post-column reagent was passed through a mixing-tee using a syringe pump. The mixed solution is measured by a UV-vis spectrophotometer through the flow cell, as shown in Figure 2. The conditions for separation and identification can be found in the literature (18, 19).

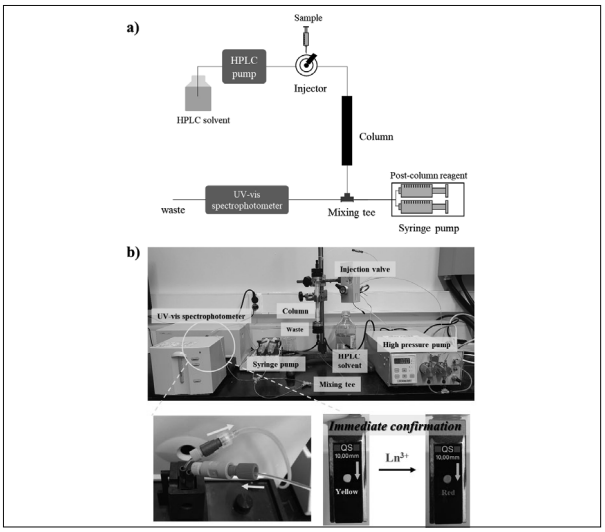

Figure 2. a) Schematic diagram and b) photographs for HPLC with post-column reaction system. The picture below shows the color change upon detection of Ln^{3+} through the flow cell.

Separation of stable isotopes Dy and Ho

$DyCl_3 \cdot 6H_2O$ (69.6 mg) and $HoCl_3 \cdot 6H_2O$ (46 mg) were added to a 10 mL vial containing 5 mL of 0.1 M HCl. The vial was heated until the solvent evaporated and 2.5 ml of 0.1 M HCl was added. This sample was stored at 4 ℃ under a nitrogen atmosphere. A portion (0.5 ml) of the sample, including 3 mg of Dy and 2 mg of Ho, was taken and injected into the prepared column A and then the flow of the eluent (0.1 M α-HIBA) was adjusted to 2.0 ml/min under an isocratic condition. In the same way, the sample to be injected into column B was prepared with $DyCl_3 \cdot 6H_2O$ (464 mg) and $HoCl_3 \cdot 6H_2O$ (23 mg). A portion (0.5 ml) of the sample contains 20 mg of Dy and 1 mg of Ho. At the same time, the post-column reagent was eluted to the mixing-tees by operating the syringe pump at a rate of 0.5 ml/min to identify the metal complexes. The mixed solution was then monitored using a UV-vis spectrophotometer at an interval of 30 s or 1 min. To assess the reproducibility of the separation, data were obtained from five independent experiments.

Separation of n.c.a ^{166}Ho

Dy_2O_3 (^{164}Dy 96.8%, 100 mg) target material was added in a quartz tube, which was sealed with a flame torch. Neutron irradiation was done under 1 x 10^{14} flux for 24 hours at Maria reactor in Radioisotope Centre POLATOM (Poland). The irradiated target was dissolved in concentrated hydrochloric acid at 110℃ under a nitrogen atmosphere. Once the solution was evaporated to dryness, the residue was re-dissolved in 1 ml of 0.01 M HCl for separation. The column B connected to a lab-made separation LC system (Figure 3) equipped with a 6-port valve, selection valve, variable sample injector, γ-ray detector, and concentration apparatus. The sample solution (0.5 ml) was injected and then 0.1 M α-HIBA was delivered at a flow rate of 2.0 ml/min under an isocratic condition, followed by conversion to 0.15 M α-HIBA for rapid elution the target. The γ-ray of the

eluate was directly measured at the outlet of the column by a Cs/I detector protected by a lead shield.

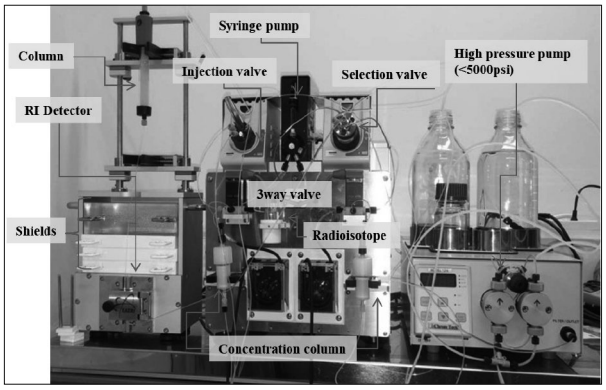

Figure 3. Photographs for lab-made radioisotope separation system.

Results and discussion

The sensitivity and specificity of the post-column reagent are important requirements for the chromogenic reaction to identify lanthanides. Thus, preliminary work was performed to evaluate a suitable reagent by investigating the chemical reaction between lanthanide and chromogenic reagents. In the cases of PAR, Arsenazo III, and Xylenol orange, the initial colors turn to red, bluish green and pink, respectively, upon chromogenic reaction with the lanthanide (Figure 1). Considering that α-HIBA is also a metal-complexing agent, it is important to note that the conditions in which lanthanides are replaced from α-HIBA to the chromogenic reagents are considered. Furthermore, rapid reaction time was required and a proper pH range should be maintained for changing their original colors. Each coupling agent forms a metal complex at a specific pH range and in the case of α-HIBA (6), PAR (11), Xylenol orange (15), and Arsenazo III (14), they form metal complexes near pH 4.2, pH 9.8, pH 5.8, and pH 2.4, respectively. At the specific pH range, PAR and Arsenazo III formed stable metal complexes with lanthanide and their colors changed to red and bluish green. However, the color of the Xylenol orange complex diminished as α-HIBA increased. This is because each pH range that forms the complex (α-HIBA: pH 4.2, Xylenol orange: pH 5.8) is adjacent to each other. Therefore, the chromogenic reaction was masked by a small amount of α-HIBA. Cupferron can be identified at the range from 270 nm to 280 nm, but their intensity of UV area is very low and is also not verified at the visible area. As a result, Xylenol orange and Cupferron cannot be used as a post-column reagent in this study.

A comparison test of two chromogenic reagents (PAR and Arsenazo III) was performed according to an increase of the metal ions. Figure 4a shows the absorption spectra of PAR at different concentrations of Ho^{3+}. PAR and PAR-Ho^{3+} complex can be identified around 415 nm and 510 nm, respectively. When the concentration of Ho^{3+} ion was increased, the band of PAR (415 nm) was reduced and the band of PAR-Ho^{3+} (510 nm) was increased. The color change to confirm the complexation reaction was immediate. Figure 4b also shows the absorption spectra of Arsenazo III at different concentrations of Ho^{3+}. Arsenazo III is identified at around 540 nm and the newly formed absorption band at 655nm is assigned to the Arsenazo III-Ho^{3+} complex. In addition, a new shoulder band could be identified near 605 nm when Ho^{3+} was higher than 25 ppm. This spectrum indicates the formation of new type metal complex and it may affect the quantitative analysis. From the qualitative analysis point of view, the absorbance of 0.1 M PAR was 0.6, while for 0.1 M Arsenazo III, it was 0.3. When 25 ppm Ho^{3+} was used, the absorbance of PAR and Arsenazo III complexes was 0.4 and 0.1. To better understand the evaluation of PAR and Arsenazo III, the absorption peaks of each reagent were plotted against the Ho^{3+} concentration (Figure 4c). For the absorbance slope according to concentration, PAR and Arsenazo III were 0.012/ppm and 0.003/ppm, respectively, and the intensity of PAR was four times greater than that of Arsenazo III. Ultimately, PAR is

favorable for analysis of trace amounts of lanthanide due to its high binding affinity and visual sensitivity.

The eluate with the separated stable isotope through the column was confirmed by a post-column reaction using PAR. As the lanthanide is eluted, the color of the mixed solution changes from yellow to red, presenting a noticeable color change (Figure 2b). Figure 5 shows the initial eluate curve for the separation of the stable isotopes Dy^{3+} and Ho^{3+} monitored by the UV-vis spectrophotometer. Research on the separation of lanthanides in a cation-exchange column using α-HIBA began 50 years ago (20). The basic principle of lanthanide separation involves the binding force with lanthanides and α-HIBA. Due to the increased atomic number in the same period, it will induce lanthanide contraction and smaller ionic radii, and eventually the complex of Ho with α-HIBA shows more thermodynamic stability than Dy. Therefore, Ho^{3+} is eluted first (6). Based on this, α-HIBA is used as an eluent and various conditions were set to obtain the nca Ho^{3+}. At pH 4.2 and RT, reducing the concentration of α-HIBA (0.12 M, 0.1 M, 0.08 M) improved the separation, but it took a long time (over 2 hrs) when 0.08 M was used, and Ho and Dy peaks overlapped when 0.12 M was used. Considering that the capacity of a general cation exchange resin is 2 meq./g, 5 g of resin (column A), corresponding to 1000 times the sample (total 5 mg, 0.01 meq), was used. At a flow rate of 2 ml/min, optimal lanthanide (Dy and Ho) separation was achieved with a maximum internal pressure of 250 psi. The internal pressure of the column (YMC, Eco Plus Glass Column) is limited to below 797 psi.

When checking the metal complex during separation, some background noise occurs between the eluate curve of Ho^{3+} and Dy^{3+} due to the staining effect of the tube by PAR, which can be ignored. The separation resolution (*Rs*) provides a quantitative assessment of its ability to separate two adjacent lanthanides. Since chromatographic curves are generally assumed to be Gaussian in shape, the resolution is defined as the difference in retention times (t_R) over the sum of peak widths (w), and can be obtained via the following equation.

$$R_S = \frac{2(t_{R,Dy} - t_{R,Ho})}{w_{Ho} + w_{Dy}} \quad (1)$$

For Figure 5a, the retention times of Ho ($t_{R,Ho}$) and Dy ($t_{R,Dy}$) are 15.5 min and 26 min, respectively, and the separation resolution is approximately 1.24. The reproducibility results are shown in Table 1. The mean value of t_R, Ho and t_R, Dy is 15.9 ± 0.29 min and 26.7 ± 0.51 min, respectively, and that of separation resolution (*RS*) is 1.4 ± 0.08, resulting in good reproducibility for t_R and *Rs*. Also, it is shown that the column is reusable. It is difficult to carry out a reproducibility evaluation within a short period by measuring with other analytical techniques such as AAS. Therefore, these results suggest that on-line detection through the post-column reaction makes it possible to obtain various information such as separation results, column reuse, and reproducibility.

Figure 5b shows that a separation between 1 mg of Ho and 20 mg of Dy was achieved when using column B. The retention times of Ho ($t_{R,Ho}$) and Dy ($t_{R,Dy}$) are about 66 min and 113 min, respectively, and the separation resolution is approximately 3.03, indicating that the two metals can be sufficiently separated. It is noteworthy that Dy^{3+} and Ho^{3+} were separated within a total separation time of 2 hours despite using large amount (20 mg) of Dy.

The chromatographic parameters found by the post-column reaction system were applied to the separation of the radioisotope ^{166}Ho. Figure 6 shows the eluate curve for the separation of ^{166}Ho from the irradiated Dy_2O_3 target monitored by a Cs/I detector. The retention times of the radioisotopes Ho^{3+} and Dy^{3+} are about 60.5 min and 90.5 min, respectively, and the separation resolution

is approximately 1.46. Under separation conditions of 0.1 M HIBA (pH 4.2 adjusted with NH_4OH), flow rate of 2.0 ml/min, and 1.0 x16 cm column, a medically important nca ^{166}Ho was separated from a 100 mg of Dy_2O_3 target. Although further experiments are required to obtain quantitative data, the present results indicate that it is useful to first find the optimal parameters of stable isotope separation and then to apply them to radioisotope separation.

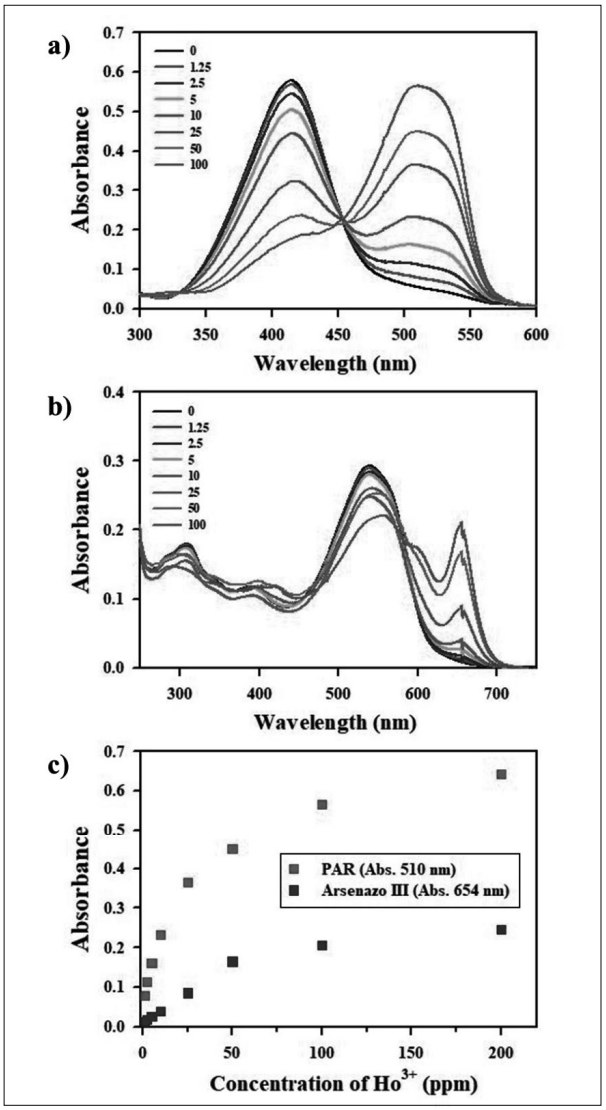

Figure 4. UV-Vis absorption spectra of a) PAR and b) Arsenazo III in the presence of varied concentration (ppm) of free Ho^{3+} ion in buffer solution. c) Absorbance of the new absorption band of PAR (red) and Arsenazo III (blue) against the concentration of Ho^{3+}.

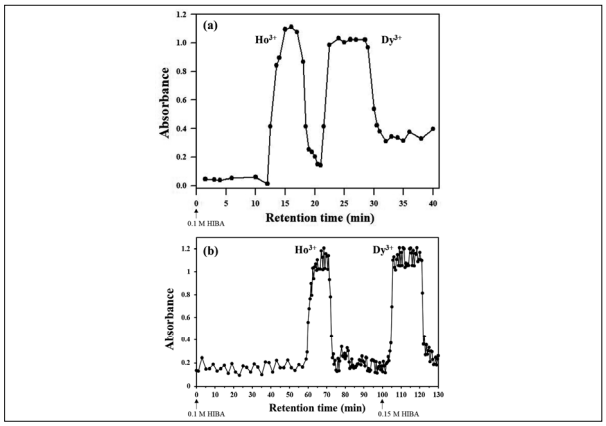

Figure 5. The eluate curve for separation of stable isotope Dy and Ho monitored by a UV-vis spectrophotometer using chromogenic reaction with PAR. (a) Ho 2 mg and Dy 3 mg load on column A (1.0 x 5.5 cm) and (b) Ho 1 mg and Dy 20 mg load on column B (1.0 x 16 cm).

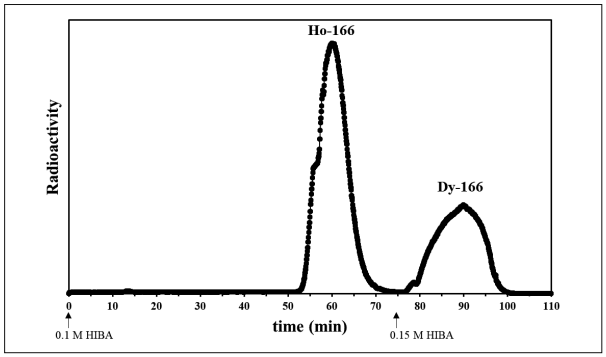

Figure 6. The separation of ^{166}Ho from the irradiated Dy_2O_3 target.

Table 1. Retention times (t_R), peak widths (w), and separation resolution (R_S) of the five different measurements for reproducibility of column

No.	$t_{R,Ho}$ (min)	$t_{R,Dy}$ (min)	w_{Ho} (min)	w_{Dy} (min)	*Rs*
1	15.5	26	7	10	1.24
2	16	27.25	6	9.5	1.45
3	16	27	6	10	1.38
4	15.75	26.35	6.5	9.3	1.34
5	16.25	26.85	6.5	9.3	1.34

Conclusion

Radioisotopes of lanthanide series have been contributing to a paradigm shift from diagnostic to therapeutic technology in the nuclear medicine fields. In order to evaluate the separation of radioisotopes, in general, an experimental assessment is done with radioisotopes themselves, which causes contamination and a large

amount of radioactive waste. Since radioisotopes also have a unique physical characteristic called half-life, rapid separation and production systems within a limited time are required. Considering these requirements, it is thought that evaluation with a stable isotope is necessary. On-line detection of metal ions using chromogenic reagent, which has been used in limited research applications such as determining the metal contents in rocks and soil, can be an alternative. Although not much research has incorporated this technology into the production of nca radioisotopes, it is expected to contribute to the optimization of production in the near future. In these studies, it was confirmed that PAR is a suitable reagent for detecting lanthanides, and this technique can be applied to separate adjacent metal-radioisotopes.

Acknowledgments

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