Production of no-carrier-added Ho-166 for targeted therapy purposes

Original Article

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ABSTRACT

Introduction: Holmium-166 radionuclide is one of the most effective radionuclides used for targeted therapy with theranostic properties. One method to produce this radioisotope is *via* the decay of its parent (indirect method). In this study applicability of extraction chromatography (EXC) for separation of no carrier added ^{166}Ho from neutron-irradiated natural dysprosium target followed by quality control procedures have been demonstrated.

Methods: ^{166}Dy was produced by thermal neutron bombardment (5×10^{13}n/cm^2.s) of natural ^{164}Dy target through ^{164}Dy (n,γ) ^{165}Dy (n,γ) ^{166}Dy process in a nuclear reactor. The generator-produced ^{166}Ho was separated from ^{166}Dy by extraction chromatographic method. The extractant used in resin was 2-ethylhexyl 2-ethylhexylphosphonic acid (HEH[EHP]). The final solution went through radionuclide, chemical and radiochemical purity tests.

Results: Using 1.5 M HNO_3 as eluent at 25 °C, and flow rate of 1.5 mL/min, quantitative separation between Ho and Dy was achieved using LN2 resin in 1.5 h to yield in no carrier added $^{166}HoCl_3$ (radionuclide purity >99.9%; separation yield; 76% and radiochemical purity >99% ITLC).

Conclusion: High specific activity $^{166}HoCl_3$ produced in this study is highly suitable for metal sensitive labeling of monoclonal antibodies, fragments and especially peptides to yield efficient therapeutic doses for human applications.

Key words: Dy-166/Ho-166; No-carrier–added; Quality control; Extraction chromatography

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INTRODUCTION

Holmium-166 is one of the most interesting radionuclides for targeted therapy modalities because of its proper physical properties, which include high energy beta radiation ($E_{\beta1}$=1854.9 keV (50%), $E_{\beta2}$=1774.3 keV (48%), $E_{\beta,ave}$=670keV), long penetration range in soft tissue (8.7mm), proper half-life 26.6 h and decay to a stable daughter [1]. Holmium-166 also emits low-intensity and low-energy γ-rays (80.5 keV, 6%) which are suitable for imaging. ^{166}Ho can be prepared by two different methods, a direct neutron capture using a ^{165}Ho target (natural abundance=100%) [2] and an indirect method *via* the ^{164}Dy (n,γ) ^{165}Dy (n,γ) ^{166}Dy(β decay) ^{166}Ho.

The direct method contains a large amount of carrier holmium atoms leading to low specific activity however could be successfully used in the development of small molecules for bone marrow ablation [3, 4]. Although many Ho-166 radiolabeled antibodies have been reported using direct method [5], however usually for therapeutic doses of monoclonal antibodies and peptides, the use of high specific activity is favored.

On the other hand, due to physical transformation of ^{166}Dy to ^{166}Ho in a suitable metal-complexing agent attached to a targeting ligand, possible *in vivo* generator possessing therapeutic potential can be expected, a similar methodology has been reported for ^{62}Zn/^{62}Cu system [6].

In this experiment applicability of extraction chromatography (EXC) for separation of NCA ^{166}Ho from neutron-irradiated natural dysprosium target, ($Dy(NO_3)_3.5H_2O$), has been demonstrated. The ion exchange systems have solubility problems and the recovery of the lanthanide from the eluent requires further processing. As the method described in herein need no recovery of the lanthanide from the eluent. The quality control of final $^{166}HoCl_3$ solution is also reported.

METHODS

Reagents

Natural $Dy(NO_3)_3.5H_2O$ was obtained from Alfa Aesar Co. LN2 resin (25-53µm particle size) and DGA resin (50-100µm particle size) were purchased from Eichrom. Nitric and hydrochloric acid were supplied from Merck Company. All other chemicals were of analytical grade. Radio-chromatography was performed by counting of ITLC-SG and/or Whatman papers using a thin layer chromatography scanner, Bioscan AR2000 (Paris, France). ITLC-SG was purchased from UK. Other chemicals were purchased from Sigma-Aldrich. A high purity p-type coaxial HPGe detector coupled with a CanberraTM (model GC1020-7500SL) multichannel analyzer with a relative efficiency of 80 % was used for gamma spectrometry. Length and diameter of the crystal were about 69 and 65 mm, respectively. Used detector well shielded and calibrated using three point-like sources of ^{152}Eu,^{133}Ba and ^{137}Cs manufactured by Amersham Company.

Irradiation

^{166}Dy was produced by double neutron capture of the natural $Dy(NO_3)_3.5H_2O$. Natural dysprosium is composed of seven isotopes of natural dysprosium: ^{156}Dy(0.06%), ^{158}Dy(0.1%), ^{160}Dy(2.34%), ^{161}Dy(18.9%), ^{162}Dy(25.5%), ^{163}Dy(24.9%), and ^{164}Dy(28.2%). One milligram of natural $Dy(NO3)_3.5H_2O$ containing ^{164}Dy (28.2%), was irradiated in the Research Reactor of Tehran at a thermal neutron flux of 5×10^{13} $ncm^{-2}s^{-1}$ for 7 days. Irradiated natural dysprosium using neutrons produces a large number of radionuclides such as Dy-157 ($T_{1/2}$=8.14 h), Dy-159 ($T_{1/2}$=144.4 d), Dy-165 ($T_{1/2}$=2.33 h), Dy-165m ($T_{1/2}$=1.25 min), Dy-166 ($T_{1/2}$=81.6 h), Dy-167 ($T_{1/2}$=6.2 min), Dy-168 ($T_{1/2}$=8.7 min), Ho-166 ($T_{1/2}$=26.6 h), Ho-166m ($T_{1/2}$=1.2E3 y), Ho-167 ($T_{1/2}$=3.003 h) and Ho-168 ($T_{1/2}$=2.99 m) through (n,γ) reactions. After 48h, the remaining radionuclides are Dy-159, Dy-166, Ho-166 and Ho-166m. Consequently, the irradiated target was allowed to decay for 48 h, then, HNO_3 (1100 µl, 0.05 M) was added to it and stirred for 1 min. For radionuclidic purity, 100 µl of the prepared solution was checked using HPGe spectroscopy. An activity of approximately 3.12 mCi of ^{166}Dy and 5.4 mCi of ^{166}Ho was produced.

Separation of ^{166}Ho from ^{166}Dy

Extraction chromatography (EXC) is a separation strategy that combines the selectivity of solvent extraction with the ease of operation and rapidity of a column chromatography. In this study, the extractant used in resin was 2-ethylhexyl 2-ethylhexylphosphonic acid (HEH[EHP]) (Figure 1).

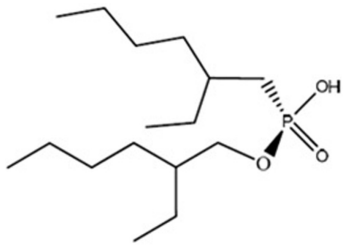

Fig 1. Chemical formulas HEH[EHP] used in this work for extraction chromatography.

LN2 resin, was made from (2-ethyl-1-hexyl) phosphonic acid mono (2-ethyl-1-hexyl) ester (HEH[EHP]) and an inert polymeric substrate (Amberchrom CG71m). In a typical preparation procedure, 10 g of purified HEH[EHP] was dissolved in 25 mL of methanol and mixed with 15 g of 25–53 mm particle size Amberchrom and stirred for 1 h on a rotary evaporator. The methanol was removed by applying a vacuum and heating the mixture to 50 °C using a water bath to yield a free flowing material of 40 wt% loading of HEH[EHP] on Amberchrom. The DGA resin was prepared in a similar manner, using tetra(1-octyl)-3-oxapentane-1,5-diglycolamide (TODGA) and 50–100 mm particle size Amberchrom. Both resins are available from Eichrom Technologies, Inc. as LN2 resin, F-grade, narrow particle size (HEH[EHP] material) and DGA resin, normal (TODGA material) [7].

The following columns was prepared for separation of ^{166}Dy/^{166}Ho by Extraction chromatography:

Column A: A 1.1 x 21 cm glass column was packed with 20-50 mesh of LN2 resin and was operated at 25 or 50 ºC using a recirculating water.

Column B: A glass column (with a dimensions of 0.1 x 10 cm) was packed with DGA resin (50-100 mesh) and was operated at room temperature.

To achieve the optimal performance of extraction chromatography, before packing, about 10 g of LN2 resin was wetted by 0.1N nitric acid for 24h. The suspension of resin was carefully added to columns with the end capped (no flow). Once the height of the column approached the desired bed height, the column was uncapped and allowed to flow. Additional suspension of resin was added until the column was slightly reached the desired bed height (20 cm) and then a layer of glass wool was inserted as a top bed support. For passing solutions through columns, a peristaltic pump and a connected polyethylene tubes was used. Column was pre equilibrated with respectively 50 cc of distilled water, 30 cc of 0.1N HNO_3. The irradiated target was loaded on the column A at a flow rate of 2 ml/min, was washed with 0.1N HNO_3 and was eluted with 1.5N HNO_3. The eluted solution was collected in 5 ml bed volume and analyzed for Dy and Ho radionuclide using the HPGe detector. The 1379 keV gamma rays of ^{166}Ho and 426 keV gamma rays of ^{166}Dy were used for detection. To adjust the solution acidity and the purification of ^{166}Ho from metal ions, DGA resin was applied in the next step. The collected solution containing of ^{166}Ho in pervious step was loaded onto the column B (DGA resin) and washed well with (HNO_3 30 mL, 0.1 M). The Purified 166Ho was eluted with HCl (50 mL, 0.05 N).

Radiochemical purity

The mixture was filtered through a 0.22 µm filter (Millipore, Millex GV) for possible use in the radiolabeling step. The radiochemical purity of the $^{166}HoCl_3$ was checked using two solvent systems for instant thin layer chromatography (ITLC) (A: 10mM DTPA pH.4 and B: ammonium acetate 10%:methanol [1:1]. In additional, to chek amount of cold Dy, a sample of product was analyzed by ICP Mass Spectrometer after deay of radioactivity.

RESULTS AND DISCUSSION

Direct method of Ho-166 production results in carrier-added product with very low specific activity. In indirect method, ^{166}Dy can be prepared by double thermal neutron capture reaction using a ^{164}Dy target: ^{164}Dy (n,γ)^{165}Dy (n,γ)^{166}Dy and decays to ^{166}Ho (Figure 2).

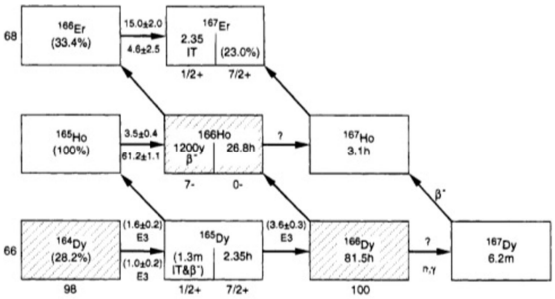

Fig 2. Scheme for production of ^{166}Dy and ^{166}Ho in a nuclear reactor [8].

The produced No-Carrier-Added (NCA) ^{166}Ho has higher specific activity compared to the direct method. (The specific activity of NCA ^{166}Ho is almost four times higher than carrier added ^{166}Ho).

The indirect method is based on initial chemical separation of the daughter radionuclide from the parent radionuclide. The reversed phase ion-exchange chromatography method based on the cation- exchange resins such as Dowex AG 50W and Aminex-A5 and sodium or ammonium salts of α-hydroxyisobutyric acid (α-HIBA) as complexing agents, has been used for separation of rare earth metals [9, 10]. The separation of several rare earth metals has been achieved by a cation exchange resin IEX-210SC [11]. Wofatit KPS cation exchanger and Tiron (disodium salt of pyrocatechol-3,5-disulfonic acid) as eluent have been applied for the purification of yttrium from heavy lanthanides (mainly Dy and Ho). Dynamic ion-exchange chromatography [12], partition chromatography using tributyl phosphate (TBP) as the stationary phase and 11-15M HNO_3 or HC1 as the mobile phase [13, 14], electrophoresis on

paper and acetylcellulose films in a medium of α-HIBA [15], high-voltage capillary electrophoresis [16] and solvent extraction techniques [17, 18] have also been used for separations of rare earth metals.

In spite of the rather large number of publications on rare earth separations, there are few reports on the separation of No-Carrier-Added (NCA) ^{166}Ho from Dy_2O_3 targets. Dadachova et *al.* used metal-free HPLC system with Dowex AG 50WX12 or Aminex-A5 cation exchangers and α-HIBA as the eluent (0.085 M, pH = 4.3 adjusted with NH_4OH) to achieve quantitative separation of NCA ^{166}Ho from neutron irradiated dysprosium targets. The overall radiochemical yield for carrier-free ^{166}Ho through an Aminex-A5 column was 95% with Dy breakthrough of <0.1% [19]. They also achieved a partial separation of NCA ^{166}Ho from dysprosium oxide target using partition chromatography and electrophoresis methods in 1995. Stationary phase of chromatography and complexing agent of electrophoresis were HDEHP or TBP and α-HIBA respectively. This group has found that electrophoresis technique was rather unsuitable for quantitative separation of NCA ^{166}Ho from milligram quantities of Dy target but partial separation was achieved by partition chromatography method [1]. Nayak et *al.* separated carrier-free ^{160}Ho-^{157}Dy from the bulk target matrix europium by utilizing liquid-liquid extraction (LLX) and HDEHP, but they could not separate ^{160}Ho from ^{157}Dy due to their similar chemical properties [20]. Successful separation of NCA ^{166}Ho from the bulk dysprosium target has been achieved by HPLC using AminexA7 ion exchanger resin and α-HIBA as the mobile phase. Lahiri et *al.* used both Dowex-50 and Aminex-A7. They have reported that no separation was observed with Dowex 50, while NCA ^{166}Ho can be achieved with Aminex-A7. This separation was quantitative and without any contamination from the dysprosium target [21].

EXC is a separation strategy that combines the selectivity of solvent extraction with the ease of operation and rapidity of a column chromatography. Horwitz et *al.* investigated the separation of ^{177}Lu/^{176}Yb by EXC resin containing 2-ethylhexyl 2-ethylhexylphosphonic acid (HEH[EHP]) absorbed onto a 25–53 mm Amberchrom CG-71 substrate [22].

Dysprosium-166 is produced by double neutron-capture on ^{164}Dy in a nuclear reactor.

After irradiation of the $Dy(NO_3)_3.5H_2O$ it was dissolved in 0.05N HNO_3 (1ml). This solution was contained Dy-159, Dy-166, Ho-166 and Ho-166m (Ho-166/Ho-166m ratio was 8.88).This solution was injected over the column A. The column was then washed with 30 ml HNO_3 (0.1N) for removing impurities. After washing, elution was carried with 200 ml HNO_3 (1.5N). Fractions of 5ml were collected and analyzed by gamma spectrometry using an HPGe detector.

The result of separation of ^{166}Ho/^{166}Dy mixture was shown in Figure 3. ^{166}Ho separation yield of 76% has been achieved. All separation process took about 1.5 hour.

To adjust the solution acidity, after gamma spectrometry analysis, fractions containing of 166Ho were passed through the column B (DGA resin).

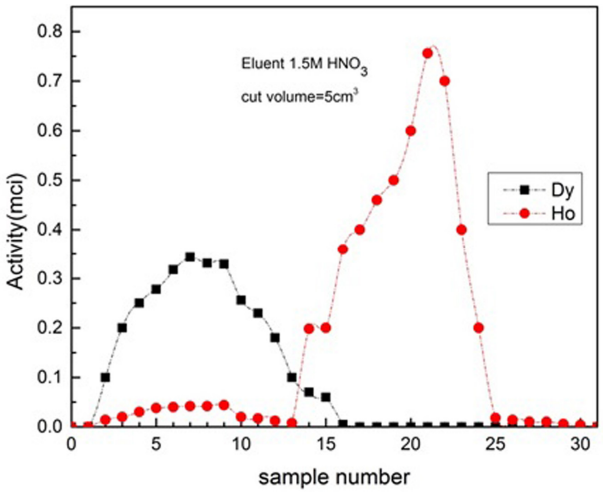

Fig 3. Separation of Ho and Dy on LN2 Resin at 25 °C, 20–50 µm particle size, 19 mL bed volume, 20 cm bed height, 1.1 cm column diameter, flow rate 1.5 mL/min.

Figure 4 illustrates a gamma ray spectra of the irradiated target and the final product, as well.

For radiochemical purity two solvent systems were used (Figure 5). In 10% NH_4OAc:methanol mixture (1:1) mixture the free cation remains at the base while any undistinguished anions would migrate to higher R_fs (not observed). On the other hand in 10 mM DTPA solution Ho-166 cation is complexed in ^{166}Ho-DTPA form migrating to higher R_fs and any possible colloidal fraction would remain at the base. The differences of impurity peaks in the two chromatograms could be related to the presence of colloidal impurity (less than 1%).

Finally, amount of cold Dy in final solution was checked by ICP mass spectrometer analysis after decay of holmium. The result of this analysis indicated that there was no cold Dysprosium in final solution.

CONCLUSION

The separation process was described here is based on extraction chromatography method, where Ho and Dy were separated by passing eluent through column containing LN2 resin.

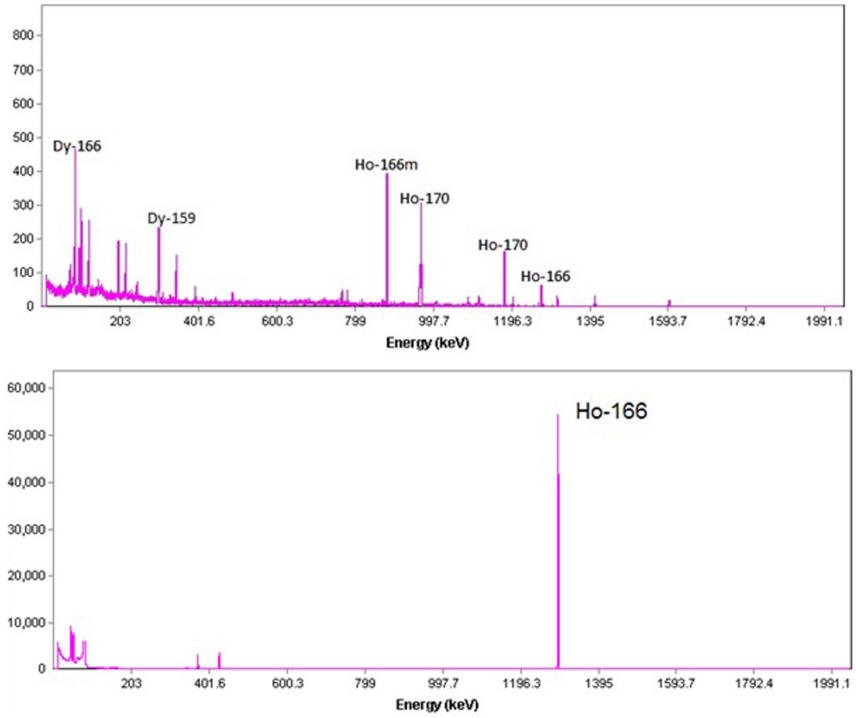

Fig 4. The gamma ray spectra of irradiated target (above) and the final product after separation (below).

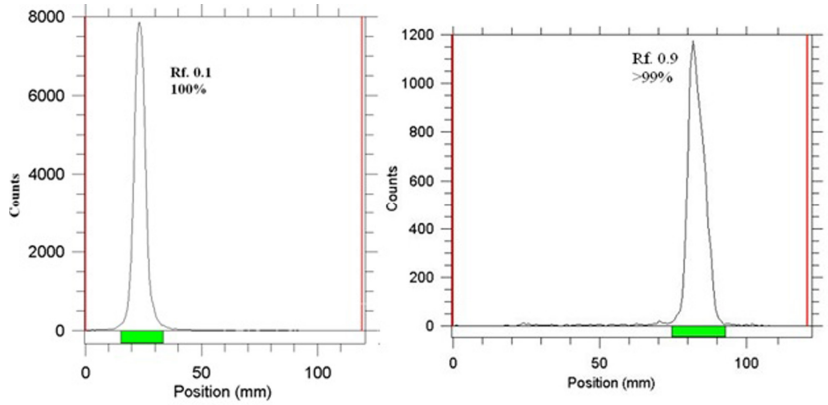

Fig 5. ITLC chromatograms of $^{166}HoCl_3$ in 10 mmol.L-1 DTPA aq. solution (right), and 10% ammonium acetate:methanol mixture (1:1) (left) at optimized conditions.

The ^{166}Dy being eluted first from the column and followed by ^{166}Ho. In addition to, this method didn't require the recovery of the lanthanide from the eluent. Under conditions of 1.5 M HNO_3 (as an eluent), T = 25 °C, and flow rate of 1.5 mL/min, quantitative separation between Ho and Dy was achieved in a column containing LN2 resin (1.1 x 21 cm, 20-50mesh). In this study, no carrier added ^{166}Ho was obtained with radionuclide purity >99.9% and overall separation yield of 76% and in >99% radiochemical purity using ITLC. The entire separation process takes about 1.5h.

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REFERENCES

1. Firestone RB, Shirley VS, Lawrence Berkeley Laboratory. Table of isotopes, 8E. CD-ROM. John Wiley & Sons, Inc. http://www.wiley.com/legacy/products/subject/physics/toi/
2. Bagheri R, Jalilian AR, Bahrami-Samani A, Mazidi M, Ghannadi-Maragheh M. Production of Holmium-166 DOTMP: A promising agent for bone marrow ablation in hematologic malignancies. Iran J Nucl Med. 2011;19(1):12-20.
3. Fakhari A, Jalilian A R, Yousefnia H, Bahrami Samani A, Akbari MR, Johari Deha F, Shafiee-Ardestani M, Khalaj A. Production, quality control, biodistribution assessment and preliminary dose evaluation of 166Ho-alendronate as a bone marrow ablative agent. Radiochim Acta. 2015;103(11):789-797.
4. Yousefnia H, Zolghadri S, Jalilian AR, Tajik M, Ghannadi-Maragheh M. Preliminary dosimetric evaluation of (166)Ho-TTHMP for human based on biodistribution data in rats. Appl Radiat Isot. 2014 Dec;94:260-5.
5. Khorami-Moghadam A, Bolouri B, Jalilian AR, Bahrami-Samani NM, Mazidi SM, Alirezapour B. Preclinical evaluation of holmium-166 labeled anti-VEGF-A(Bevacizumab). J Labelled Comp Radiopharm. 2013 Jun 30;56(8):365-9.
6. Gholipour N, Jalilian AR, Fazaeli Y, Sabzevari O, Moradkhani S, Bolourinovin F, Khalaj A. Development of [62Zn/62Cu]-DOTA-rituximab as a possible novel in vivo PET generator for anti-CD20 antigen imaging. Radiochim Acta. 2014;102(11):1035–1045.
7. Horwitz EP, McAlister DR, Bond AH, Barrans RE, Williamson JM. A process for the separation of 177Lu from neutron irradiated 176Yb targets. Appl Radiat Isot. 2005 Jul;63(1):23-36.
8. Knapp Jr FF. Reactor-produced therapeutic radionuclides. In: Knapp Jr FF, Dash A. Radiopharmaceuticals for therapy. Springer; 2016. p. 71-113.
9. Smith HL, Hoffman DC. Ion-exchange separations of the lanthanides and actinides by elution with ammonium alpha-hydroxy-isobutyrate. J Inorg Nucl Chem. 1956 Oct;3(3-4):243-7.
10. Choppin GR, Chopoorian JA. Complexes of the lanthanide elements with α-hydroxy carboxylate ligands. J Inorg Nucl Chem. 1961 Dec; 22(1-2):97-113.
11. Yoshida K, Haraguchi H. Determination of rare earth elements by liquid chromatography/inductively coupled plasma atomic emission. Anal Chem. 1984 Nov;56(13):2580-5.
12. Barkley DJ, Blanchette M, Cassidy RM, Elchuk S. Dynamic chromatographic systems for the determination of rare earths and thorium in samples from uranium ore refining processes. Anal chem. 1986 Sep;58(11):2222-6.
13. Mirzadeh, S. ORNL Nuclear Medicine Program Progress Report. ORNL/TM-12159. 1992; 6-11.
14. Sochacka RJ, Siekierski S. Reversed-phase partition chromatography with di-(2-ethylhexyl) orthophosphoric acid as the stationary phase: Part I. Separation of rare earths. J Chromatogr. 1964 Nov;16:376-84.
15. Aitzetmüller K, Buchtela K, Grass F, Hecht F. Electrophoresis of rare earth elements on cellulose acetate. Mikrochim Acta. 1966;(6):1101-13.
16. Chen M, Cassidy RM. Bonded-phase capillaries and the separation of inorganic ions by high-voltage capillary electrophoresis. J Chromatogr A. 1992 Jun 5;602(1-2):227-34.
17. Tang J, Wai CM. Solvent extraction of lanthanides with a crown ether carboxylic acid Anal Chem. 1986 Dec;58(14):3233-5.
18. Stevenson PC, Nervik WE. The radiochemistry of the rare earths: scandium, yttrium, and actinium. Washington: National Academies; 1961.
19. Dadachova E, Mirzadeh S, Lambrecht RM, Hetherington EL, Knapp Jr FF. Separation of carrier-free holmium-166 from neutron-irradiated dysprosium targets. Anal Chem. 1994 Dec;66(23):4272-7.
20. Nayak D, Lahiri S, Ramaswami A, Manohor SB. Separation of carrier-free holmium and dysprosium produced in 70 MeV 11 B 5+ irradiated europium target by liquid-Liquid extraction with HDEHP. Indian J Chem. 2000;39(10):1061-1065.
21. Lahiri S, Volkers KJ, Wierczinski B. Production of 166Ho through 164Dy(n, gamma)165Dy(n, gamma)166Dy(beta-)166Ho and separation of 166Ho. Appl Radiat Isot. 2004 Dec;61(6):1157-61.
22. Horwitz EP, Bloomquist CA. Chemical separations for super-heavy element searches in irradiated uranium targets. J Inorg Nucl Chem. 1975;37(2):425-34.