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Tandem column isolation of zirconium-89 from cyclotron bombarded yttrium targets using an automated fluidic platform: Anion exchange to hydroxamate resin columns

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ABSTRACT

The development of a tandem column purification method for the preparation of high-purity ^{89}Zr(IV) oxalate is presented. The primary column was a macroporous strongly basic anion exchange resin on styrene divinylbenzene co-polymer. The secondary column, with an internal volume of 33 μL, was packed with hydroxamate resin. A condition of inverted selectivity was developed, whereby the ^{89}Zr eluent solution for the primary column is equivalent to the ^{89}Zr load solution for the secondary column. The ability to transfer ^{89}Zr from one column to the next allows two sequential column clean-up methods to be performed prior to the final elution of the ^{89}Zr(IV) oxalate. This approach assures delivery of high purity ^{89}Zr product and assures a ^{89}Zr product that is eluted in a substantially smaller volume than is possible when using the traditionally-employed single hydroxamate resin column method. The tandem column purification process has been implemented into a prototype automated fluidic system. The system is configured with on-line gamma detection so column effluents can be monitored in near-real time. The automated method was tested using seven cyclotron bombarded Y foil targets. It was found that 95.1 ± 1.3% of the ^{89}Zr present in the foils was recovered in the secondary column elution fraction. Furthermore, elution peak analysis of several ^{89}Zr elution profile radiochromatograms made possible the determination of ^{89}Zr recovery as a function of volume; a ^{89}Zr product volume that contains 90% of the mean secondary column elution peak can be obtained in 0.29 ± 0.06 mL (representing 86 ± 5% of the ^{89}Zr activity in the target). This product volume represents a significant improvement in radionuclide product concentration over the predominant method used in the field. In addition to the reduced ^{89}Zr product elution volume, titrations of the ^{89}Zr product with deferoxamine mesylate salt across two preparatory methods resulted in mean effective specific activity (*ESA*) values of 279 and 340 T Bq·mmole^{-1} and mean bindable metals concentrations ($[M_B]$) of 13.5 and 16.7 nmole·g^{-1}. These *ESA* and $[M_B]$ values infer that the ^{89}Zr(IV) oxalate product resulting from this tandem column isolation method has the highest purity reported to date.

1. Introduction

Zirconium-89 (^{89}Zr), a positron emitting radionuclide ($t_{1/2}$ = 78.41 h), produced from the (*p, n*) reaction with naturally monovalent yttrium-89 (^{nat}Y), is gaining a use for Positron Emission Tomography (PET) based diagnostic imaging. It has been the subject of several recent reviews [1–6]. Since the Meijs et al. team reported the use of a hydroxamate resin column for ^{89}Zr isolation in 1994 [7], the purification of ^{89}Zr for PET imaging applications has been based almost exclusively on this approach. This method has been demonstrated to be capable of producing a ^{89}Zr product that contains ≥ 85% [7–11] of the column-loaded activity in a 1–2 mL fraction using 1 M oxalic acid ($H_2C_2O_4$) as an eluent.

Until recently, the ^{89}Zr isolation method has been performed manually. However, automated fluidic systems are beginning to emerge in this field [9,11–13]. O'Hara et al. [13] recently reported an automated ^{89}Zr purification system that was programmed to perform a modified version of the hydroxamate resin column method; it was demonstrated that a lower concentration (0.8 M) of $H_2C_2O_4$

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was adequate for efficient ^{89}Zr elution, with 90% of the ^{89}Zr elution profile being delivered in 1.02 ± 0.09 mL. This product volume corresponded to 84 ± 2% of the original ^{89}Zr activity in the bombarded foil.

Reports on the quality of ^{89}Zr products, purified by the prevailing hydroxamate resin column method [8,10], have been sparse in the literature, as surveyed by Wooten et al. [12]. Effective specific activity (*ESA*) is the ratio of isotope product activity to the mole quantity of isotope product contaminants that compete for the chelate conjugated onto the protein; these contaminants can adversely impact protein radiolabeling yield [14–16]. Holland et al. [8] reported an *ESA* of 17.4–44.2 T Bq·mmol^{-1} (470–1195 Ci·mmol^{-1}), while Wooten et al. [12], using an automated purification system, reported 0.2–13.1 T Bq·mmol^{-1} (5–353 Ci·mmol^{-1}). More recently, Lin et al. [9] and O'Hara et al. [13] reported *ESA* values of 75.48–158.36 T Bq·mmol^{-1} and 44 ± 7 and 109 ± 22 T Bq·mmol^{-1}, respectively, using fluidic systems.

Unfortunately, *ESA* is not a good comparative measure of radionuclide product quality, as it is dependent on the activity of the radionuclide produced in the cyclotron target, and does not consider the cyclotron target mass from which the ^{89}Zr was produced. Therefore, differences in *ESA*s between products of varied ^{89}Zr activity and/or target mass do not provide a comparable measure of product quality.

This team [13] reported the bindable metals concentration ($[M_B]$) of the ^{89}Zr product fraction, a term that is independent of the activity present in the isotope product. $[M_B]$ is a measure of the mole quantity of metals (e.g., ^{89}Zr + elemental Zr, Fe, etc.) present in the isotope product that compete with a radiolabeling chelate, normalized to the mass of Y present in the originating cyclotron target. The ^{89}Zr product fractions generated by the automated system employing the modified hydroxamate resin column method was reported as between 43 ± 7 and 115 ± 27 nmole·g^{-1}. These values were similar to the approximated $[M_B]$ resulting from the Holland et al. report (60-150 nmol·g^{-1}) [8], which was derived from peripheral information provided in the article (an equation to approximate $[M_B]$, based on activity at end of bombardment, *ESA* and target mass, was presented in the earlier report [13]).

In an effort to determine whether improvements in ^{89}Zr product purity were possible beyond that provided by the traditional hydroxamate resin column method, our team evaluated the use of a tandem column separation method, wherein ^{89}Zr could be purified in two stages. To establish a tandem column separation, chemical conditions must be determined whereby selectivity transfer occurs. In this case, selectivity transfer between two columns occurs when the elution condition for a product ion on the primary column is equivalent to a loading condition of the product ion on the secondary column. By fluidically coupling the outlet of the primary column to the inlet of the secondary column during the primary column elution step, the analyte can be effectively transferred to the secondary column. The purification of medical isotopes using a similar product transfer strategy has been demonstrated [17,18].

Selectivity transfer is distinct from that used in a Multi-Column Selectivity Inversion Generator, as defined and demonstrated by Horwitz, Bond, and McAlister [19–22]. Using this approach, an adsorbed product ion elutes from a primary column and passes unretained through a secondary (guard) column, while residual contaminant ions are trapped on the secondary column. As such, the isotope product volume is determined by the elution volume required to strip the isotope from the primary column, plus additional dispersion of the elution band caused by the guard column. In contrast, selectivity transfer enables the primary column eluent to be concentrated on a secondary column of significantly smaller volume in relation to the primary column, since the complex solution matrix (e.g., dissolved target metal) has been removed prior to the transfer step. The small secondary column, in turn, allows for a subsequent purification stage of the targeted isotope, and results in elution of the twice-purified isotope in a reduced volume, thereby increasing the isotope concentration.

We recently described the optimization of ^{89}Zr uptake and elution from a macroporous, strongly basic anion exchange resin (MP-1 M) column using automated fluidics [23]. The work asserted that the optimal Zr eluent solution was 6 M HCl containing 0.33 mM NaF; the addition of a minute quantity of fluoride ion to the eluent aided in sharpening the otherwise broad Zr elution band from the MP-1 M column. This MP-1 M column method was designed to be the first stage of ^{89}Zr purification in a tandem column method. In this installment, we report the use of a hydroxamate resin microcolumn (33 μL internal volume) as the secondary column in a tandem column method.

In order to design a tandem column method based on selectivity transfer, the behavior of Zr(IV) on hydroxamate resin was evaluated across a range of HCl and fluoride ion concentrations. Batch distribution coefficient (K_d) values were determined for Zr (as ^{88}Zr) for HCl concentrations between 1 and ~12 M, and for 6 M HCl with fluoride ion concentrations ranging between 0 and 1 mM. Next, the hydroxamate resin capacity was assessed to assure that a microcolumn could bind sufficient Zr to assure that breakthrough would not occur. Finally, hydroxamate resin microcolumn performance during load/wash and elute steps were evaluated using on-line detection of the microcolumn effluents delivered via a simple fluidic device.

The aforementioned assessments facilitated the development of a MP-1 M → hydroxamate resin tandem column purification method for ^{89}Zr using a prototype automated fluidic system. The objectives of this work were to: 1) demonstrate that computer-controlled fluidics can be employed to facilitate a dual-stage ^{89}Zr purification process for eventual use in remote (e.g., shielded) locations; 2) increase the purity of a ^{89}Zr product to levels beyond that achieved by the predominantly employed hydroxamate resin column method; 3) deliver a ^{89}Zr product in a reduced volume in order to increase product isotope concentration; and 4) assure the resulting ^{89}Zr(IV) oxalate product had high binding affinities via transchelation from the oxalate to the deferoxamine forms. The purity of resultant ^{89}Zr products was assessed by evaluating ^{89}Zr binding to deferoxamine mesylate (DFOM), as desferrioxamine B is the chelating ligand most commonly conjugated to monoclonal antibodies (mAbs) for ^{89}Zr-based PET imaging applications [3,4,24]. The ^{89}Zr product quality achieved via the tandem column method is reported as both *ESA* and $[M_B]$. These results are then compared to reported and derived values from available literature.

2. Experimental

2.1. Reagents, standards, and materials

TraceMetal and Optima grade hydrochloric acid (34–37% HCl, Fisher Scientific, Waltham, MA) working stock solutions were prepared as-is or as dilutions into deionized water (18.3 MΩ cm) using a Barnstead E-Pure water purification system (Dubuque, IA). Method development work and cyclotron bombarded Y foil processing utilized TraceMetal and Optima grade acids, respectively. Sodium fluoride (99.99+ %), hydrogen peroxide (30%), and TraceSELECT® oxalic acid dihydrate (> 99.9999%) were purchased from Sigma-Aldrich (St. Louis, MO). AG MP-1 M strongly basic anion exchange resin (Cl^- form, 200–400 mesh), with quaternary amine functional groups on a macroporous styrene divinylbenzene copolymer (Bio-Rad Life Sciences, Hercules, CA) was used for the primary column. Hydroxamate ligand was synthesized onto Accell Plus CM weak cation-exchanger (37–55 μm, Waters Corp., Milford,

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