Published in final edited form as:

Metallomics. 2011 December ; 3(12): 1304–1309. doi:10.1039/c1mt00114k.

Stability assessment of different chelating moieties used for elemental labeling of bio-molecules

Daniela Kretschy, Gunda Koellensperger, and Stephan Hann

University of Natural Resources and Life Sciences, BOKU Vienna, Department of Chemistry, Division of Analytical Chemistry, Muthgasse 18, A-1190 Vienna, Austria

Abstract

Integrating elemental labeling in quantitative LC-ICP-MS based bio-analysis requires fundamental experiments concerning the stability of complexes during analysis. In a competitive approach complex stability of the chelating moieties 1,4,7,10-tetraazacyclododecane-N, N', N'', N'''-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-N, N', N''-triacetic acid (NOTA) and diethylenetriaminepentaacetic dianhydride (DTPA) in combination with 11 different lanthanides was investigated under typical chromatographic conditions. Measurements were carried out via LC-ICP-QMS using a novel mixed mode separation method. The influence of chromatographic separation, pH and temperature on complex stability constants was assessed regarding further applications of multiplexing in bio-analytical assays. The limit of detection (LOD) for LC-ICP-QMS were 0.03 nM for all investigated Tm complexes (0.15 fmol absolute). Quantification of the complexes was performed via external, flow injection based calibration. For all investigated complexes the stability was significantly decreased by the chromatographic conditions. Moreover, complexation by DOTA revealed two different signals suggesting the presence of a stable intermediate product. Ln^{3+} -DOTA and Ln^{3+} -NOTA complexes provided high stability at 5 °C and 37 °C over a time of 12 hours, whereas Ln^{3+} -DTPA complexes showed significant degradation at 37 °C.

Introduction

The speciation and quantification of various bio-molecules in samples with complex matrix is a main issue in quantitative proteomics^{1,2}, metabolomics and metallomics^{3,4}. Especially labeling of bio-molecules represents an essential tool for sensitive detection and has already been applied in biological, medical and analytical sciences^{5,6,7}. It has been shown that labeling offers general advantages, i.e. increased selectivity, sensitivity and robustness in complex matrices and applicability of flow injection analysis for determination of total drug concentrations^{4,8,9,10}. In this context many different isotope selective strategies have been developed where ICP-MS shows very promising results as a sensitive detector for metal labeled bio-molecules^{11,12,13}.

A special feature of elemental labels combined with ICP-MS detection is the capability of isotope dilution analysis (IDA)^{14,15,16,17} and the option for multiplexing^{18,19}. 1,4,7,10-tetraazacyclododecane-N, N', N'', N'''-tetraacetic acid (DOTA) and diethylenetriaminepentaacetic dianhydride (DTPA) based reagents are often used as chelating moieties for labeling with lanthanides because of their high complex stability

© The Royal Society of Chemistry

Corresponding author: Stephan Hann, University of Natural Resources and Life Sciences, BOKU Vienna, Department of Chemistry, Division of Analytical Chemistry, Muthgasse 18, A-1190 Vienna, Austria; stephan.hann@boku.ac.at; phone.: +43-47654-6086; fax: +43-47654-6059.

constants, but also other cyclic ligands like 1,4,7-triazacyclononane-N, N', N''-triacetic acid (NOTA) are available^{20,21,22}. Lanthanides (Ln³⁺) are ideal metals for multiplexed labeling of bio-molecules due to their low natural abundance in biological samples and their similar chemical nature. The lanthanide series ranges from Ce to Lu (because of their similar chemistry La and Y are often included) and they differ primarily in their ionic radii, which show a decrease along the series (lanthanide contraction)²³. In the case of DOTA and NOTA, complex coordination is maintained via free electron pairs of heteroelements as well as via carboxylate groups and is mainly influenced by pH, temperature and concentration^{20,22,24,25,26}. Biomedical applications of the complexes are e.g. radio pharmaceuticals, magnetic resonance imaging contrast agents or fluorescent macrocyclic complexes^{22,26,27}.

Chromatographic separation of the different labeled bio-molecules is a prerequisite in quantitative speciation analysis. However, separation is challenging due to the similar structure of most applied labels and the lack of complex stability, which can cause problems during sample preparation and chromatographic separation. Most studies addressing the assessment of complex stability found in literature were performed at neutral pH^{20, 22, 23, 26,28}, which is limiting in terms of chromatographic method development. Therefore in this work we focused on the determination of complex stability under acidic separation conditions (pH 2.5) with identification of the most stable lanthanide complexes as a main goal. A novel chromatographic separation method for different elemental complexes was developed and applied for simultaneous assessment of complex stability of the chelating moieties with various lanthanides under the selected conditions and at different temperatures. To the best of our knowledge this is the first systematic approach addressing complex stability under experimental conditions present in LC-ICP-MS.

Experimental

Reagents and standards

For preparation of the ammonia acetate buffer (pH 6.5) applied for labeling suprapure ammonia (Merck, Darmstadt, Germany) and suprapure acetic acid (Merck, Darmstadt, Germany) was used. The ammonia formate buffer (pH 2.5), which was employed as an eluent for FIA-ICP-QMS and LC-ICP-QMS analysis was prepared using suprapure ammonia (Merck, Darmstadt, Germany) and suprapure formic acid (Merck, Darmstadt, Germany). The ammonia acetate labeling buffer (pH 6.5) was prepared using suprapure ammonia (Merck, Darmstadt, Germany) and suprapure acetic acid (Merck, Darmstadt, Germany). For wash solutions and eluents subboiled nitric acid (Merck, Darmstadt, Germany) and suprapure methanol LC-MS grade (Honeywell, New Jersey, USA) were used. Labeling ligands DOTA and NOTA were purchased at CheMatech (Dijon, France) and DTPA was purchased at Sigma-Aldrich (Steinheim, Germany). The employed 10 mg L⁻¹ multi element ICP-QMS standard solution containing a mixture of rare earth elements (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Ho, Dy, Er, Tm, Yb, Lu, Th, Y and Sc) dissolved in HNO₃ and diluted with ultra pure water (final acidity 5% HNO₃ w/w) was purchased at SPEX CertiPrep Inc.

(Metuchen, USA). Water was pre-cleaned by reverse osmosis and afterwards purified using a purification system (SG Ultra Clear

Basic, SG Wasseraufbereitung und Regeneration GmbH, Barsbüttel, Germany).

Sample preparation

Before formation of lanthanide complexes and LC-ICP-QMS analysis the chelating moieties 1,4,7,10-tetraazacyclododecane-N, N', N'', N'''-tetraacetic acid (DOTA), 1,4,7-

triazacyclononane-N, N', N''-triacetic acid (NOTA) and diethylenetriaminepentaacetic dianhydride (DTPA) were weighed and dissolved in ammonia acetate buffer (pH 6.5) resulting in a clear solution with a concentration of approximately 4 μ M. The multi element ICP-MS standard solution was diluted with ammonia acetate buffer (pH 6.5) to a concentration of approximately 0.25 μ M. Regarding water solubility DOTA and NOTA revealed a good solubility in aqueous solution whereas DTPA showed inferior solubility and needed more time to dissolve completely.

Preparation of lanthanide complexes

The procedure for preparation of the lanthanide complexes was adapted from Waentig²⁴ and Jeong et al²⁹. Accordingly, the reaction was carried out at constant ionic strength in 20 mM ammonia acetate buffer pH 6.5. The employed chelating moieties are depicted in Fig. 1. 0.25 μ M lanthanide and 4 μ M chelating moiety were incubated for 15 minutes at room temperature according to Jeong et al²⁹. Moreover, two additional time points (6 and 12 hours incubation time) were examined in order to investigate the influence of time on complex formation. Afterwards the samples were kept at 5°C or 37°C during LC-ICP-QMS analysis. The approach with 37°C auto sampler temperature was chosen to simulate labeling under physiological conditions.

Instrumental

For the speciation of the different lanthanide complexes an inert titanium HPLC gradient system (Transcend Liquid Chromatography System, Thermo Scientific, Munich, Germany) in combination with a metal-free autosampler (HTC PAL Autosampler, Thermo Fisher Scientific Inc., Waltham, USA) was combined with a ICP-QMS detector (XSERIES 2 ICP-QMS, Thermo Scientific Inc., Bremen, Germany). The used LC column was a SEC RP WAX mixed mode column (Asahipak GS 220 HQ, Shodex, Munich, Germany) with a dimension of 4.6×250mm. For direct connection of the HPLC effluent to the PFA-nebulizer (PFA-ST, Elemental Scientific Inc., Omaha, Nebraska, USA) and for all other connections PEEK capillaries (IDEX) with an internal diameter of 0.127 mm were used. The PFA-nebulizer was operated in a cooled (5°C) cyclonic silica glass spray chamber (PC³, ISA Elemental Scientific). The LC-ICP-QMS operating parameters are summarized in Table 1. The ICP-QMS was operated in standard mode without use of the collision cell. For calibration and quantification of the lanthanide complexes flow injection analysis was performed with the same system without separation column.

Data Evaluation

Aria HPLC software (Version 1.6.3., 2010, Thermo Scientific Inc., Bremen, Germany) was used for controlling the Transcend HPLC system. Plasmalab software (Version 2.5.11.321, 2007, Thermo Scientific Inc., Bremen, Germany) was used for controlling the ICP-QMS system and for the generation and export of FIA-ICP-QMS and LC-ICP-QMS chromatograms. The integration of all chromatographic data from FIA-ICP-QMS and LC-ICP-QMS analysis was carried out using Chromeleon software (Version 6.80 SR 10, Dionex, Sunnyvale, California, USA).

Quantification

For external calibration via FIA-ICP-QMS DOTA, NOTA and DTPA complexes in combination with 11 lanthanides with concentrations ranging from 12.5 to 130 nM were assessed. For each rare earth element a calibration function was calculated using Microsoft Office Excel (version 2007). A typical FIA-ICP-QMS signal of a 12.5 nM standard of Tm-DOTA is depicted in Fig. 2.

Calculation of complex binding constants

Equation 1 was adapted from the work of C. Huang and D. Beachemin³⁰ and used for calculation of complex stability constants.

$$\log K = \log \frac{[MeL]}{[Me]} - n \log c$$

K stands for the determined complex stability constant of the complex MeL between a metal ion Me and a ligand L. The chelating number of the complex is n and c stands for the total concentration of the ligand after complex stability. The complex stability constants determined in our work resulted from competitive incubation of different lanthanides with the same chelating agent, which is of special importance for future applications in multiplexed assays with differential labeled samples.

Results and discussion

Quantitative bio-analysis using elemental labeling in combination with LC-ICP-MS approaches is a topical research theme. In this context the stability of the elemental label throughout the whole analytical process is a key issue. Separation of bio-molecules is commonly performed by reversed phase, ion exchange or size exclusion chromatography with reversed phase being the predominant separation mode. Due to separation problems a combination of these methods is often performed via two dimensional chromatography. In order to circumvent multidimensional chromatography we have selected a mixed mode stationary phase providing all three separation modes and therefore ultimate selectivity regarding the separation of complex mixtures containing neutral and ionic compounds. In preliminary experiments reversed phase or ion exchange chromatographic columns did not solve this separation problem.

In a first step the stability of lanthanide complexes was assessed under typical chromatographic conditions to be later applied for analysis of labelled bio-molecules. In the present study only the influence of pH on the stability of Ln³⁺-complexes with different ligands was assessed. Future experiments will address the impact of organic solvents and reversed phase chromatography in the context of maleimide functionalized complexing moieties.

Separation of the different complexes was achieved by increasing the ionic strength of the mobile phase via gradient elution. We investigated the stability of lanthanide complexes at pH 2.5, and under these conditions free metal ions could be detected. The signals obtained for DOTA, NOTA and DTPA complexes with Tm at 5°C and 37°C are shown in Fig. 3. The peak eluting at 5.6 min retention corresponds to Tm-NOTA, and the peaks at 7 min, 7.8 min and 9.2 min correspond to Tm-DOTA, Tm-DOTA* and Tm-DTPA, respectively. The peak at 11 min corresponds to free Tm. For the investigated complexes the peak width at 50% peak height was between 0.14 – 0.30 min. The number of theoretical plates per meter was between 20,000 and 40,000 for DOTA and NOTA and between 100,000 and 130,000 for DTPA.

LC-ICP-QMS detection of DOTA, NOTA and DTPA complexes with 11 different lanthanides was performed simultaneously. The linearity of flow injection based calibration was in the range of 0.999 for all investigated rare earth elements. The obtained sensitivities were approximately 60,000 cps peak height for 12.5 nM Tm. The limit of detection (LOD) for LC-ICP-QMS were 0.03 nM for all investigated Tm complexes (0.15 fmol absolute) with an injection volume of 5 µL into a flow of 400 µL min⁻¹.

Determination of complex stability under the selected chromatographic conditions

The stability constants of complexing moieties used for elemental labeling are well known. These studies have been performed at neutral pH^{20, 22, 26, 28}. However, in LC-based bio-analytical assays an acidic pH is in many cases a prerequisite for chromatographic separation of labeled bio-molecules. Determination of complex stability constants requires quantification of metal complexes and unbound metals and therefore chromatographic separation is an important issue. The complex stability constants (expressed as log K) determined under the applied conditions are presented in Table 2. Since the selected pH determines the proteolysis degree of the chelating moieties, the complex stability constants are conditional and valid for the applied pH of 2.5. Accordingly they are significantly lower than the values found in the literature^{20, 22, 26, 28}, but in accordance to other studies addressing the impact of pH on complex stability²⁰. In the case of Ln³⁺-DOTA (see peak at 7.8 min, Fig. 3) an additional peak appears in the LC-ICP-QMS chromatogram at lower pH. We suggest that this signal corresponds to the positively charged intermediate product [Ln(III)-DOTA]⁺, which is in accordance to E. Toth et al.²⁰, who observed the formation of this product under low pH conditions. Our results show that at 5°C the ratio of Ln³⁺-DOTA to Ln³⁺-DOTA* is between 4.7 and 4.9.

In the present study the different complexing moieties revealed stabilities in the order of Ln³⁺-DOTA > Ln³⁺-NOTA >>> Ln³⁺-DTPA. An overview of the determined stability constants is given in Table 2. It is noteworthy that the suitability of DOTA as complexing moiety under the selected conditions can be hampered by the formation of the positively charged intermediate product. This is a severe drawback with respect to application of elemental labeling under the selected conditions as originally only one species is required. Regarding Ln³⁺-DTPA our results show that the investigated complexes were not stable and showed low complex stability constants indicating that DTPA is unsuitable for elemental labeling under the employed separation conditions. Ln³⁺-NOTA complexes showed higher stability under the investigated conditions, with stability constants between 0.2 and 3.1. Moreover, the obtained signals showed a single complex peak indicating that no intermediate product was formed during HPLC separations.

Additionally, our results suggest that there is a correlation between complex stability and the ionic radii of the lanthanides, which corresponds to observations made under neutral conditions²¹. As can be seen in Table 2 complexation of DOTA, NOTA and DTPA with the lanthanides Pr and Nd led to low complex stability and these metals are therefore not adequate for applications in bio-analytical assays requiring acidic conditions. However, lanthanides with ionic radii between 92 and 86 pm (Tb-Lu) are highly suitable for application in future bio-assays employing macrocycles as DOTA or NOTA for elemental labeling.

Influence of incubation time and temperature

We investigated the influence of residence time on the autosampler (i.e. incubation time at pH 6.5 in 20 mM ammonium acetate buffer) at two different temperatures (5 °C and 37 °C). More specifically three different time points (15 minutes, 6 hours and 12 hours) were examined. It can be clearly seen in Fig. 4a-c that, under the selected chromatographic conditions, the stability constants of Ln³⁺-DOTA, Ln³⁺-DOTA* and Ln³⁺-NOTA did not significantly change with increasing incubation time at both temperatures. In the case of Ln³⁺-DTPA (Fig. 4d) the constants were decreasing with time, indicating insufficient stability for the intended use in bio-analytical assays under acidic conditions. Additionally, our investigations revealed that Ln³⁺-DOTA* is present in all investigated samples independent of incubation temperature and time and that the ratio of Ln³⁺-DOTA to Ln³⁺-DOTA* is between 4.0 and 4.9 in all investigated samples.

Conclusion

Ln^{3+} -DOTA complexes exhibit highest log K values with the disadvantage that the chromatographic separation shows an additional signal suggesting a positively charged intermediate product. Log K values of Ln^{3+} -NOTA complexes are 0.2 – 3.1 for the selected lanthanides providing one single compound. The stability of Ln^{3+} -DTPA complexes is not satisfying under the selected conditions. Summarising, the developed LC-ICP-QMS method using a mixed mode stationary phase suggests NOTA as a complexing moiety for sensitive and multiplexed analysis in future bio-analytical applications, with respect to sample preparation and analysis steps at acidic conditions. It is also noteworthy that, under such non-optimal conditions, lanthanides with ionic radii between 92 – 86 pm (Tb – Lu) should be selected. As a matter of fact this is of minor importance for applications, which are performed at neutral pH or physiological conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge the Austrian Science Found (Project P21739-B11) for providing financial support and Thermo Scientific for providing the Transcend HPLC System.

Notes and references

- ‡1. Wiese S, et al. Protein labeling by iTRAQ: A new tool for quantitative mass spectrometry in proteome research. *Proteomics*. 2007; 7:340. [PubMed: 17177251]
2. Liu H, et al. Method for quantitative proteomics research by using metal element chelated tags coupled with mass spectrometry. *Anal. Chem.* 2006; 78:6614. [PubMed: 16970341]
3. Mounicou S, Szpunar J, Lobinski R. Inductive-coupled plasma mass spectrometry in proteomics, metabolomics and metallomics studies. *Eur. J. Mass Spectrom.* 2010; 16:243.
4. Jakubowski N, et al. Labeling of proteins with 2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid and lanthanides and detection by ICP-MS. *J. Anal. At. Spectrom.* 2008; 23:1497.
5. Gygi S, et al. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nat. Biotechnol.* 1999; 17:994. [PubMed: 10504701]
6. Whetstone P, et al. Element-coded affinity tags for peptides and proteins. *Bioconjugate Chem.* 2004; 15:3.
7. Ornatsky O, et al. Study of cell antigens and intracellular DNA by identification of element containing labels and metallointercalators using inductive coupled plasma mass spectrometry. *Anal. Chem.* 2006; 80:2539. [PubMed: 18318509]
8. Kretschy D, et al. High-throughput flow injection analysis of labeled peptides in cellular samples—ICP-MS analysis versus fluorescence based detection. *Int. J. Mass Spectrom.* 2011 article in press.
9. Ahrends R, et al. A metal-coded affinity tag approach to quantitative proteomics. *Mol. Cell. Proteomics*. 2007; 6:1907. [PubMed: 17627934]
10. Becker J, et al. Bioimaging of metals in brain tissue by laser ablation inductive coupled plasma mass spectrometry (LA-ICP-MS) and metallomics. *Metallomics*. 2010; 2:104. [PubMed: 21069140]
11. Prange A, Pröfrock D. Chemical labels and natural element tags for quantitative analysis of biomolecules. *J. Anal. At. Spectrom.* 2008; 23:432.
12. Hempen C, Karst U. Labeling strategies for bioassays. *Anal. Bioanal. Chem.* 2006; 384:572. [PubMed: 16208465]
13. Wang M, et al. ICP-MS-based strategies for protein quantification. *Mass Spectrom. Rev.* 2010; 29:326. [PubMed: 19492311]

14. Mann M, et al. Functional and quantitative proteomics using SILAC. *Nat. Rev. Mol. Cell Biol.* 2006; 7:952. [PubMed: 17139335]
15. Tholey A, Schaumlöffel D. Metal labeling for quantitative protein and proteome analysis using inductively-coupled plasma mass spectrometry. *Trends Anal. Chem.* 2010; 29:399.
16. Schaumlöffel D, Lobinski R. Isotope dilution technique for quantitative analysis of endogenous element species in biological systems. *Int. J. Mass Spectrom.* 2005; 242:217.
17. Bettmer J. Application of isotope dilution ICP-MS techniques to quantitative proteomics. *Anal. Bioanal. Chem.* 2010; 397:3495. [PubMed: 20532485]
18. Lou X, et al. Polymer based elemental tags for sensitive bioassays. *Angew. Chem. Int. Ed.* 2007; 46:6111.
19. Waenig L, et al. Multi-parametric analysis of cytochrome P450 expression in rat liver microsomes by LA-ICP-MS. *J. Anal. At. Spectrom.* 2011; 26:310.
20. Toth E, et al. Kinetics of formation and dissociation of lanthanide (III)-DOTA complexes. *Inorg. Chem.* 1994; 33:4070.
21. Chacheris W, Nickel S, Sherry A. Thermodynamic study of lanthanide complexes of 1,4,7-Triazacyclononane-N, N', N''-triacetic acid and 1,4,7,10-Tetraazacyclododecane-N, N', N'', N'''-tetraacetic acid. *Inorg. Chem.* 1987; 26:958.
22. Kubicek V, Toth E. Design and function of metal complexes as contrast agents in MRI. *Adv. Inorg. Chem.* 2009; 61:63.
23. Nicholson K, Wood S. Aqueous geochemistry of rare earth elements and Yttrium. XII: Potentiometric-stability constant determination of bis-tris complexes with La, Nd, Eu, Gd, Yb, Dy, Er, Lu and Y. *J. Sol. Chem.* 2001; 31:703.
24. Waentig, L. Strategien für das Multielement-Labeling von Proteinen und Antikörpern für LA-ICP-MS-Anwendungen in der Proteomik. Technische Universität Dortmund; 2010. Dissertation
25. Kubicek V, et al. Gallium (III) complexes of DOTA and DOTA-monoamide: Kinetic and thermodynamic studies. *Inorg. Chem.* 2010; 49:10960. [PubMed: 21047078]
26. Mewis R, Archibald S. Biomedical applications of macrocyclic complexes. *Coord. Chem. Rev.* 2010; 254:1686.
27. Bonnet C, Toth E. Towards highly efficient, intelligent and bimodal imaging probes: Novel approaches provided by lanthanide coordination chemistry. *Comptes Rendus Chimie.* 2010; 13:700.
28. Zheng L, et al. Quantification of proteins using lanthanide labeling and HPLC/ICP-MS detection. *J. Anal. At. Spectrom.* 2011; 26:1233.
29. Jeong J, et al. Preparation of promising angiogenesis PET imaging agent: ⁶⁸Ga-labeled c(RGDyK)-isothiocyanatobenzyl-1,4,7-triazacyclononane-1,4,7-triacetic acid and feasibility studies in mice. *J. Nucl. Med.* 2008; 49:830. [PubMed: 18413379]
30. Huang C, Beauchemin D. A simple method based on IC-ICP-MS to determine conditional stability constants. *J. Anal. At. Spectrom.* 2006; 21:317.

Figure 1.
Molecular structure of the employed complexes.

Figure 2.

Flow injection ICP-MS signal of 3 repetitive blank injections and 12.5 nM Tm-DOTA in ammonia formate buffer with pH 2.5 (injection volume 5 μ L, absolute amount 62.5 fmol). The flow injection procedure was applied for quantification of the lanthanide complexes via external calibration.

Figure 3.

Separation of lanthanide complexes and free lanthanides obtained via LC-ICP-MS utilizing a mixed mode separation method under the conditions describes in table xx. The signals were obtained for three different solutions containing 4 μM chelating moiety (DOTA, NOTA or DTPA) and 0.25 μM lanthanide standard solution in ammonia formate buffer with pH 2.5. Tm-DOTA* corresponds to a positively charged intermediate product formed under the selected pH.

Figure 4a.

Complex formation constants ($\log K$) of Ln^{3+} -DOTA* complexes with 4 μM DOTA and 0.25 μM lanthanide standard solution containing all lanthanides and Y and Sc in ammonia formate buffer with pH 2.5. Incubation was performed at 5 °C (left) and 37° (right) with an incubation time of 15 minutes, 6 hours and 12 hours.

Figure 4b.

Complex formation constants ($\log K$) of Ln^{3+} -DOTA* complexes with 4 μM DOTA and 0.25 μM lanthanide standard solution containing all lanthanides and Y and Sc in ammonia formate buffer with pH 2.5. Incubation was performed at 5°C (left) and 37°C (right) with an incubation time of 15 minutes, 6 hours and 12 hours.

Figure 4c.

Complex formation constants ($\log K$) of Ln^{3+} -NOTA complexes with 4 μM NOTA and 0.25 μM lanthanide standard solution containing all lanthanides and Y and Sc in ammonia formate buffer with pH 2.5. Incubation was performed at 5°C (left) and 37°C (right) with an incubation time of 15 minutes, 6 hours and 12 hours.

Figure 4d.

Complex formation constants ($\log K$) of Ln^{3+} -DTPA complex with $4 \mu\text{M}$ DTPA and $0.25 \mu\text{M}$ lanthanide standard solution containing all lanthanides and Y and Sc in ammonia formate buffer with pH 2.5. Incubation was performed at 5°C (left) and 37°C (right) with an incubation time of 15 minutes, 6 hours and 12 hours.

Table 1

Operation parameters for LC-ICP-MS and FIA-ICP-MS.

Carrier flow	Ammonia formate (pH 2.53)
Flow rate	0.4 mL min ⁻¹ (0.1 mL min ⁻¹ for FIA)
Injection volume	5 µL
Tray - temperature	5 or 37 °C
Column	Asahipak GS 220 HQ 4.6×150mm
Gradient	0-50mM in 18 min, wash step with 95mM and 10 min reconditioning
Nebulizer	PFA-ST microconcentric
Spray chamber	Cyclonic, 5 °C
Nebulizer gas flow	0.86 L min ⁻¹
Auxillary gas flow	0.70 L min ⁻¹
Plasma gas	13.00 L min ⁻¹
ICP RF power	1400 W
Scan mode	Peak hopping
m/z measured	⁴⁵ Sc, ⁸⁹ Y, ¹³⁹ La, ¹⁴⁰ Ce, ¹⁴¹ Pr, ¹⁴⁴ Nd, ¹⁵⁰ Sm, ¹⁵² Eu, ¹⁵⁷ Gd, ¹⁵⁹ Tb, ¹⁶² Dy, ¹⁶⁵ Ho, ¹⁶⁷ Er, ¹⁶⁹ Tm, ¹⁷³ Yb, ¹⁷⁵ Lu;
Dwell time per isotope	0.01 s
Data points s ⁻¹	5
Cycle time	30.5 min (1.5 min for FIA)

Table 2

Logarithm of complex binding constants (log K) at 5°C and 37°C with ionic radii (pm) of all investigated lanthanides under selected conditions (sample preparation with ammonia acetate buffer pH 6.5, chromatography with ammonia formate buffer pH 2.5, gradient 0 to 50 mM) [average of 3 time points].

	log K at 5°C	log K at 37°C	Ionic radii of Me ³⁺ (pm)
Pr Nota	0.48	−0.08	99
Pr Dota	0.79	1.10	
Pr Dota*	0.04	0.28	
Pr DTPA	−0.9	−1.1	
Nd Nota	0.45	0.17	98
Nd Dota	0.72	1.03	
Nd Dota*	−0.03	0.22	
Nd DTPA	−0.43	−0.68	
Sm Nota	0.71	0.56	96
Sm Dota	1.3	1.8	
Sm Dota*	0.48	0.82	
Sm DTPA	0.10	−0.23	
Gd Nota	0.66	0.66	94
Gd Dota	1.25	1.8	
Gd Dota*	0.44	0.78	
Gd DTPA	0.08	−0.23	
Tb Nota	1.10	1.27	92
Tb Dota	2.1	3.0	
Tb Dota*	1.3	1.9	
Tb DTPA	0.18	−0.11	
Dy Nota	1.3	1.7	91
Dy Dota	2.2	3.1	
Dy Dota*	1.4	1.9	
Dy DTPA	0.16	−0.12	
Ho Nota	1.17	1.8	90
Ho Dota	2.0	2.9	
Ho Dota*	1.19	1.7	
Ho DTPA	0.10	−0.20	
Er Nota	1.22	2.0	89
Er Dota	1.9	2.7	
Er Dota*	1.02	1.6	
Er DTPA	−0.05	−0.27	
Tm Nota	1.5	2.6	88
Tm Dota	2.0	2.9	
Tm Dota*	1.11	1.7	
Tm DTPA	−0.11	−0.36	
Yb Nota	1.9	3.1	87

	log K at 5°C	log K at 37°C	Ionic radii of Me ³⁺ (pm)
Yb DOTA	2.0	2.9	
Yb DOTA*	1.11	1.7	
Yb DTPA	−0.15	−0.41	
Lu DOTA	1.5	2.9	86
Lu DOTA*	1.4	2.1	
Lu DOTA*	0.60	1.02	
Lu DTPA	−0.24	−0.53	