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Short communication

Determination of trace levels of Dy^{3+} in Dy(HP-DO3A) by ion-pair liquid chromatography with post-column reaction

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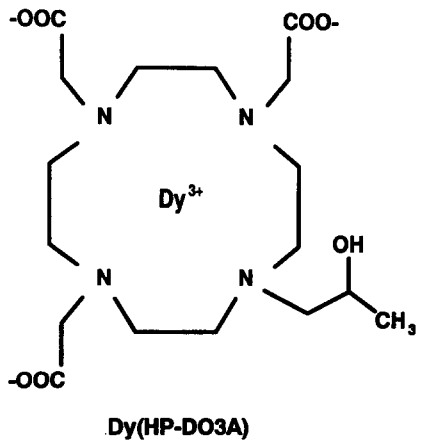
1. Introduction

It has been shown that non-ionic, gadolinium chelates, for example, gadoteridol [Gd(HP-DO3A)], are clinically useful as contrast agents in magnetic resonance imaging (MRI) [1,2]. The macrocyclic poly(amino carboxylate), 10-2-hydroxypropyl)-1, 4, 7, 10-tetracyclododecane-1,4,7-triacetic acid, HP-DO3A, forms a complex with gadolinium with a formation constant, K_f, of 23.8 [3]. The analogous dysprosium chelate, Dy(HP-DO3A) (Fig. 1), is also being evaluated as a potential MRI contrast agent which promotes T_2-shortening.



Dy(HP-DO3A)

Fig. 1. Chemical structure of the dysprosium chelate, Dy(HP-DO3A).

Since gadolinium and dysprosium are both lanthanides and Dy(HP-DO3A) is isostructural with Gd(HP-DO3A), the formation constants of Dy(HP-DO3A) and Gd(HP-DO3A) are expected to be of a similar magnitude. It is well established that free lanthanides are poorly tolerated in animals and are deposited in tissues, especially liver and bone [4,5]. It is, therefore, important to determine trace levels of free dysprosium in Dy(HP-DO3A) if this complex is to be utilized as an in-vivo contrast agent.

Since its introduction in 1975, ion chromatography has become an extremely useful method for the separation of many types of ions [6]. Ion-exchange chromatography has been especially useful for the determination of trace rare earth elements in industrial and geological materials with such detection methods as inductively coupled plasma-atomic emission spectrometry [7–9] and direct-current plasma emission spectroscopy [10,11].

In some cases, particularly for the separation of inorganic ions, ion-pair [12–16] or dynamic ion-exchange [17,18] chromatography is a viable alternative to ion-exchange chromatography. The most common mode of ion-pair chromatography is reverse phase with a mobile phase consisting of an aqueous buffer with an added counterion or ion-pair reagent.

Even with an effective separation mechanism, however, conventional LC detection systems are not well suited for the direct determination of trace levels of many metal

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ions, including lanthanides. The sensitivity and selectivity of conventional detectors may be greatly enhanced through the use of post-column reaction techniques in which the metal ions are complexed or derivatized with a chemical reagent prior to detection. Several reviews have been written detailing the advantages and disadvantages of the four major post-column reactor types: tubular or capillary, bed, segmented stream, and membrane [19–24]. Of these, membrane reactors, which deliver post-column reagent through the wall of the membrane by pressurized diffusion, offer an advantage over the other reactor types by eliminating the peak broadening and baseline noise effects often encountered when an additional pump is necessary to deliver reagent. Post-column reaction techniques with hollow fiber membrane reactors have been used for assays of penicillins [25–27], amino acids [28], barbiturates [29], β-lactamase inhibitors [30, 31], and the determination of glucose [32,33], peroxides [34], and lanthanides [35].

Combining the techniques of ion-exchange or ion-pair LC with a variety of post-column reactors and post-column reagents has led to the selective separation and sensitive detection of metal ions in a wide variety of samples [17,36–40], including lanthanides [41–43] and rare earth elements [44,45].

This paper describes the combined use of ion-pair chromatography for the separation of the lanthanide Dy^{3+} from an organic matrix with the post-column reaction of Dy^{3+} with Arsenazo III in a hollow fiber membrane reactor and detection in the visible region. Octanesulfonate was used as the ion-pair reagent to modify the reverse-phase surface of the column and allow for separation via ion-exchange. Hydroxyisobutyric acid was used as a complexing agent in the mobile phase because it has previously demonstrated excellent complexing capabilities with many polyvalent metal ions [17,38–40,42,44]. The peak shape, sensitivity, and reproducibility of standard injections were dependent on the concentration and flow rate of the post-column reagent. The linearity and limit of detection were determined.

2. Experimental

2.1. Materials

1-Octanesulfonic acid, sodium salt (octane sulfonate), 2-hydroxyisobutyric acid (HIBA), 2,2′(1,8-dihydroxy-3,6-disulfonaphthalene-2,7-bisazo)bisbenzenearsonic acid (Arsenazo III) and urea were obtained from Aldrich (Milwaukee, WI, USA). Sodium hydroxide pellets were obtained from Fisher (Springfield, NJ, USA). Trace metal grade glacial acetic acid was obtained from Mallinckrodt (Paris, KY, USA). Dysprosium chloride, hexahydrate, 99.99%, was obtained from Johnson Matthey/Aesar (Seabrook, NH, USA). The water was LC/organic free from a SYBRON/Barnstead NANOpure II system (Boston, MA, USA).

The LC system consisted of a Model 8800 pump from Spectra-Physics (Piscataway, NJ, USA) with metal-free solvent inlet filters, a Rheodyne Model 7010 injector with a titanium, 20-µl (nominal) fixed loop from Rainin Instrument Co., Inc. (Woburn, MA, USA), and an ABI Analytical Spectroflow 757 absorbance detector (658 nm) from ABI Applied Biosystems (Foster City, CA, USA).

Along with the Spectra-Physics Model 4200 integrator, the output signal was also delivered to a Spectra-Physics LABNET data collection system for processing. A Delta-Pak HPI C_{18}, 5 µm, 300 Å, 150 × 3.9 mm column from Waters, Division of Millipore (Milford, MA, USA) was used with a mobile phase containing octanesulfonate (10 mM) and HIBA (60 mM), pH 5.5, at a flow rate of 1.0 ml min^{-1}. The post-column reaction system consisted of a Dionex Reagent Delivery Module with an IonPac® membrane reactor, 48 cm fiber, from Dionex Corporation (Marlton, NJ, USA) used with a post-column reagent containing Arsenazo III (0.065 mM), urea (5 mM) and glacial acetic acid (31 mM) delivered at 0.6 ml min^{-1}, 68–72 psi. Wherever possible, the systems were plumbed with PEEK tubing.

2.2. Methods

Prior to use, the column was conditioned by pumping mobile phase at 1.0 ml min^{-1} for 0.5 h, with the column eluant flowing directly through the post-column reactor fiber. Post-column reagent was then delivered into the membrane reactor at 0.6 ml min^{-1} to permeate the fiber and mix with the column eluant. The system was ready for use when a steady baseline was obtained, usually within 0.5 h. A

Table 1
Reproducibility of Dy^{3+} standard injection as a function of the flow rate of Arsenazo III (0.13 mM)

Flow rate (ml min^{-1})	Theoretical Dy^{3+} conc. (μg ml^{-1})	Measured Dy^{3+} conc. (μg ml^{-1})	RSD (%) ($n = 10$)
0.3	6.02	6.02 ± 0.75	12.5
0.4	6.02	6.02 ± 0.58	9.6
0.5	6.17	6.17 ± 0.35	5.6
0.6	5.87	5.87 ± 0.18	3.1
0.7	6.12	6.12 ± 0.45	7.3
0.8	6.12	6.12 ± 0.31	5.0

Table 2
Reproducibility of Dy^{3+} standard injection as a function of the flow rate of Arsenazo III at a flow rate of 0.6 ml min^{-1}

Arsenazo III conc. (mM)	Theoretical Dy^{3+} conc. (μg ml^{-1})	Measured Dy^{3+} conc. (μg ml^{-1})	RSD (%) ($n = 10$)
0.130	5.87	5.87 ± 0.18	3.1
0.065	6.12	6.12 ± 0.22	3.7
0.033	6.12	6.13 ± 0.17	2.8
0.033	5.95	6.00 ± 0.23	3.8
0.013	5.95	5.94 ± 0.18	3.0

0.016 mg ml^{-1} solution of dysprosium chloride prepared in mobile phase was injected for system suitability. The RSD of the Dy^{3+} peak response was < 5%.

Working sample solutions were prepared by dissolving 60 mg of sample in 2 ml of mobile phase. To minimize dissociation and elevated levels of Dy^{3+} over time, samples were injected within 1 h of preparation. Dysprosium chloride standard solutions (0.052–14 μg Dy^{3+} ml^{-1}) were also prepared in mobile phase and were stable for 24 h.

3. Results and discussion

Ion-pair chromatography is useful for the separation of trace levels of Dy^{3+} from an organic matrix. However, Dy^{3+} does not absorb or fluoresce strongly enough to be detected by conventional LC detectors. When Dy^{3+} is complexed with Arsenazo III in a hollow fiber post-column reactor, the detector response at 658 nm is greatly enhanced. Arsenazo III exhibited a significantly higher sensitivity for Dy^{3+} than 4-(2-pyridylazo)-resor- cinol monosodium salt (PAR) which has often been used as a post-column reagent for the detection of lanthanides [37–39,41,43,45]. In this case, PAR was found to be an unsuitable reagent for several reasons including poor sensitivity and reproducibility of the Dy^{3+} peak, drifting and unstable baselines, and the observation of interfering peaks.

When post-column reactors are used with LC, peak shape, sensitivity and reproducibility are dependent on the flow rate and concentration of the post-column reagent [46]. In this case, the LC/post-column reaction system was metal-free wherever possible because of the extreme sensitivity of Arsenazo III to many free metals. Ten replicate injections of a Dy^{3+} standard (6 μg ml^{-1}) made at each of six different flow rates of Arsenazo III (0.13 mM) demonstrated that reproducibility is a function of flow rate (Table 1). A flow rate of 0.6 ml min^{-1} yielded the lowest RSD (3.1%) and, hence, the optimum flow rate of Arsenazo III in terms of reproducibility. Ten replicate injections of a Dy^{3+} standard (6 μg ml^{-1}) made at each of four different concentrations of Arsenazo III at the optimum flow rate of 0.6 ml min^{-1}) showed that, in this case, the reproducibility is not significantly effected by the concentration of post-column reagent (Table 2). However, a sharp reduction in peak tailing was observed at concentrations ⩽ 0.065 mM, with a corresponding reduction in sensitivity as the concentration was reduced. Overall, optimum peak shape, sensitivity and reproducibility are exhibited when using Arsenazo III (0.065 mM) at a flow rate of 0.6 ml min^{-1} and, under these conditions, ten replicate injections of a Dy^{3+} standard

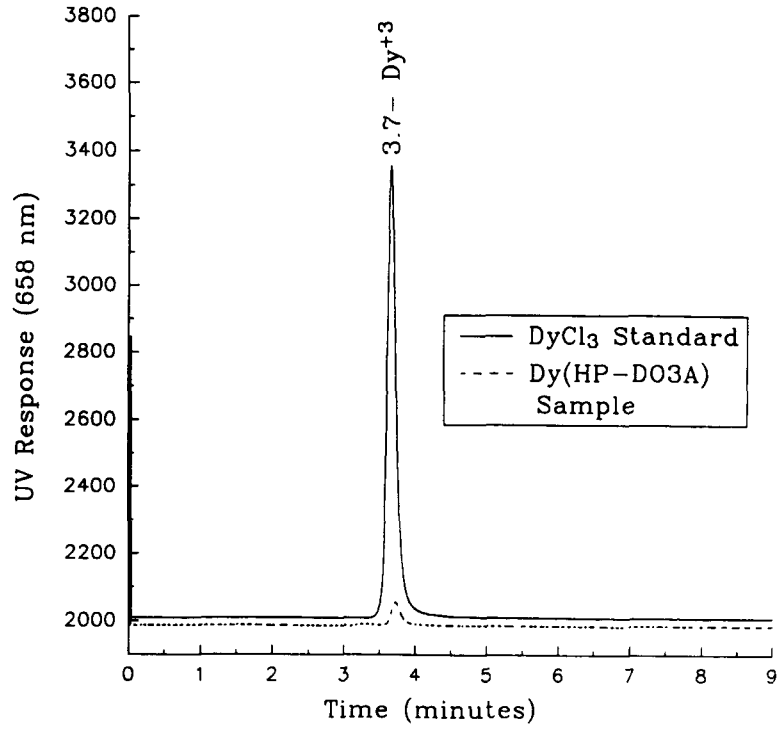

Fig. 2. Representative chromatograms of Dy^{3+} in a $DyCl_3$ standard solution (——) and a Dy(HP-DO3A) sample, 0.012% (w/w) (– – –). Conditions: Delta-Pak HPI (150 × 3.9 mm) column, aqueous mobile phase (pH 5.5) containing sodium octanesulfonate (10 mM) and hydroxyisobutyric acid (HIBA; 60 mM), at a mobile phase flow rate of 1.0 ml min^{-1}, Arsenazo III (0.065 mM) post-column reagent at a post-column reagent flow rate of 0.6 ml min^{-1} and visible detection at 658 nm.

Table 3
Retention time and respose factor of Dy^{3+} standard injection as a function of the concentration of HIBA with mobile phase pH of 3.8

HIBA conc. (mM)	Dy^{3+} retention time (min)	Dy^{3+} conc. (μg ml^{-1})	Dy^{3+} peak area counts	Dy^{3+} response factor (× 10^{-6})
100	2.5	0.77	92688	8.3
90	5.0	0.74	76458	9.7
85	6.7	0.70	81932	8.5
80	7.0	0.80	86261	9.3
75	8.4	0.69	76279	9.0

(7 μg ml^{-1}) yielded an RSD of 1.5%. A chromatogram of a standard injection is shown in Fig. 2.

The standard response was linear in the range of 0.052–14 μg Dy^{3+} ml^{-1} and linear regression analysis yielded a correlation coefficient of 0.9998. Although a placebo was not available for the determination of standard recovery, recoveries of 94.1–97.1% were obtained for a sample of Dy(HP-DO3A) spiked with 3–9 μg Dy^{3+} ml^{-1} (3–9 p.p.m.). Concentrations of Dy^{3+} as low as 0.0003% (w/w) were quantitated with $S/N \geqslant 3$ and the detection limit of Dy^{3+} in Dy(HP-DO3A) was 0.00015% (w/w). A chromatogram of an injection of a Dy(HP-DO3A) sample containing 0.0012% Dy^{3+} (w/w) is shown in Fig. 2. Ruggedness testing indicated that over 300 injections could be made on the column with no detectable reduction in column performance.

It is known that when using ion-pair chromatography, retention times can be strongly influenced by the pH of the mobile phase [16,17,43]. During method development, duplicate injections of a Dy^{3+} standard (0.7 μg ml^{-1}) made with varying concentrations of HIBA in the mobile phase (pH 3.8) showed that the retention time was also dependent

Table 4
Dissociation of a Dy(HP-DO3A) sample prepared in mobile phase (pH 5.5) as a function of time

Injection time (min)	Dy^{3+} conc. (% w/w)	Dy(HP-DO3A) dissociation (%)	Dissociation attributed to mobile phase pH (%)
0	0.00038	0.0013	NA
11	0.00043	0.0015	0.0002
21	0.00042	0.0015	0.0002
32	0.00045	0.0016	0.0003
43	0.00046	0.0016	0.0003
54	0.00054	0.0019	0.0006

upon the concentration of HIBA. However, the mM concentration of HIBA in 10 000-fold excess did not significantly effect the Dy^{3+} peak response factor, indicating that the formation of the Dy^{3+}-Arsenazo III complex was also uneffected (Table 3). For this method, retention time was not a major concern, but the pH of the mobile phase was a crucial factor in the accurate detection of Dy^{3+} owing to the potential instability of Dy(HP-DO3A) below pH 4.4. Dy(HP-DO3A) samples prepared in mobile phase with pH 5.5–7.0, when reacted with the Arsenazo III solution (pH < 3), resulted in reduced peak response and significant peak tailing. Samples prepared in mobile phases with pH < 5.5 dissociated fairly rapidly, resulting in the detection of elevated levels of Dy^{3+}. Studies were conducted to quantify the stability of the Dy(HP-DO3A) complex over time using the optimized mobile-phase conditions. Dy(HP-DO3A) sample solutions in mobile phase (pH 5.5) were analyzed for Dy^{3+} over a 1 h time period. Results revealed that no significant dissociation of the sample was measurable, other than that attributed to Dy^{3+} initially present in the sample (Table 4). Thus, Dy(HP-DO3A) samples prepared in mobile phase with pH 5.5 yielded the optimum peak response and represented the best compromise between the peak tailing observed above pH 5.5 and the sample dissociation observed below pH 5.5. When prepared with mobile phase of pH 5.5 and injected within 1 h of preparation, the peak shape is acceptable and the percentage of dissociation is not significant.

In conclusion, this ion-pair LC method combined with post-column reaction provided for the sensitive detection of Dy^{3+} in Dy(HP-DO3A), whereby concentration levels as low as 0.0003% (w/w) were easily quantitated. The method is rugged, rapid (< 10 min), linear and reproducible.

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References

[1] V.M. Runge, D.Y. Gelblum, M.L. Pacetti, F. Carolan and G. Heard, Radiology, 177 (1990) 393–400.
[2] W.T.C. Yuh, J.D. Engelken, M.G. Muhonen, N.A. Mayr, D.J. Fisher and J.C. Ehrhardt, AJNR, 13 (1992) 335–345.
[3] K. Kumar, C.A. Chang, L.C. Francesconi, D.D. Dischino, M.F. Malley, J.Z. Gougoutas and M.F. Tweedle, Inorg. Chem., 33 (1994) 3567–3575.
[4] P.W. Durbin, M.H. Williams, M. Gee, R.H. Newman and J.G. Hamilton, Proc. Soc. Exp. Biol. Med., 91 (1956) 78–85.
[5] T.J. Haley, J. Pharm. Sci., 54 (1965) 663–670.
[6] H. Small, T.S. Stevens and W.C. Bauman, Anal. Chem., 47 (1975) 1801–1809.
[7] P.S. Murty and R.M. Barnes, J. Anal. At. Spectrom., 1 (1986) 145–148.
[8] J.A. Tielrooy, P.H.M. Vleeschhouwer, J.C. Kraak and F.J.M.J. Maessen, Anal. Chim. Acta, 207 (1988) 149–159.
[9] K. Yoshida and H. Haraguchi, Anal. Chem., 56 (1984) 2580–2585.
[10] F.W.E. Strelow and A.H. Victor, Talanta, 37 (1990) 1155–1161.
[11] F. Flavelle and A.D. Westland, Talanta, 33 (1986) 445–447.
[12] B.A. Bidlingmeyer, S.N. Deming, W.P. Price, Jr., B. Sachok and M. Petrusek, J. Chromatogr., 186 (1979) 419–434.
[13] I. Molnàr, H. Knauer and D. Wilk, J. Chromatogr., 201 (1980) 225–240.
[14] J.H. Knox and R.A. Hartwick, J. Chromatogr., 204 (1981) 3–21.
[15] L.R. Snyder, J.L. Glajch and J.J. Kirkland, Practical HPLC Method Development, Wiley, New York, 1988.
[16] B.A. Bidlingmeyer, J. Chromatogr. Sci., 18 (1980) 525–539.
[17] R.M. Cassidy and M. Fraser, Chromatographia, 18 (1984) 369–373.

[18] C.P. Terweij-Groen, S. Heemstra and J.C. Kraak, J. Chromatogr., 161 (1978) 69–82.
[19] R.S. Deelder, A.T.J.M. Kuijpers and J.H.M. van den Berg, J. Chromatogr., 255 (1983) 545–561.
[20] U.A.Th. Brinkman, Chromatographia, 24 (1987) 190–200.
[21] U.A.Th. Brinkman, J. Chromatogr., 492 (1989) 251–298.
[22] H. Englehardt, R. Klinkner and G. Schöndorf, J. Chromatogr., 535 (1990) 41–53.
[23] S. van der Wal, J. Liq. Chromatogr., 6 (1983) 37–59.
[24] J.C. Davis and D.P. Peterson, Anal. Chem., 57 (1985) 768–771.
[25] J. Haginaka, J. Wakai, H. Yasuda, T. Uno, K. Takahashi and T. Katagi, J. Chromatogr., 400 (1987) 101–111.
[26] J. Haginaka and J. Wakai, J. Chromatogr., 413 (1987) 219–226.
[27] J. Haginaka, J. Wakai, Y. Nishimura and H. Yasuda, J. Chromatogr., 447 (1988) 365–372.
[28] J. Haginaka and J. Wakai, J. Chromatogr., 396 (1987) 297–305.
[29] J. Haginaka and J. Wakai, J. Chromatogr., 390 (1987) 421–428.
[30] J. Haginaka, J. Wakai and H. Yasuda, Anal. Chem., 59 (1987) 324–327.
[31] J. Haginaka, J. Wakai, H. Yasuda, T. Uno and T. Nakagawa, J. Liq. Chromatogr., 8 (1985) 2521–2534.
[32] D. Pilosof and T.A. Nieman, Anal. Chem., 54 (1982) 1698–1701.
[33] N.L. Malavolti, D. Pilosof and T.A. Nieman, Anal. Chem., 56 (1984) 2191–2195.
[34] H. Hwang and P.K. Dasgupta, Anal. Chem., 58 (1986) 1521–1524.
[35] R.M. Cassidy and S. Elchuk, Anal. Chem., 59 (1987) 85–90.
[36] R.M. Cassidy and S. Elchuk, Anal. Chem., 54 (1982) 1558–1563.
[37] R.M. Cassidy and S. Elchuk, J. Chromatogr. Sci., 18 (1980) 217–223.
[38] S. Elchuk and R.M. Cassidy, Anal. Chem., 51 (1979) 1434–1438.
[39] C.H. Knight, R.M. Cassidy, B.M. Recoskie and L.W. Green, Anal. Chem., 56 (1984) 474–478.
[40] R.M. Cassidy, S. Elchuk, N.L. Elliot, L.W. Green, C.H. Knight and B.M. Recoskie, Anal. Chem., 58 (1986) 1181–1186.
[41] A. Mazzucotelli, A. Dadone, R. Frache and F. Baffi, J. Chromatogr., 349 (1985) 137–142.
[42] G.J. Sevenich and J.S. Fritz, Anal. Chem., 55 (1983) 12–16.
[43] W. Wang, Y. Chen and M. Wu, Analyst, 109 (1984) 281–286.
[44] J. Hwang, J. Shih, Y. Yeh and S. Wu, Analyst, 106 (1981) 869–873.
[45] J.A. Tielrooy, J.C. Kraak and F.J.M.J. Maessen, Anal. Chim. Acta, 176 (1985) 161–174.
[46] Y.T. Shih and P.W. Carr, Anal. Chim. Acta, 167 (1985) 137–144.