Determination of lanthanides in synthetic standards by reversed-phase high-performance liquid chromatography with the aid of a weighted least-squares regression model
Estimation of method sensitivities and detection limits

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Abstract

A reversed-phase liquid chromatography method was used for determining lanthanides in synthetic standards. The separation of lanthanide group was achieved in less than 15 min using a linear gradient program of α-hydroxyisobutyric acid eluent from 0.05 to 0.5M (pH 3.8) with a UV–Vis detection system at 658 nm. A post-column reagent of Arsenazo III was employed for improving the sensitivity and selectivity of the method as well as for lowering the limits of detection (LODs). Linear calibration curves for all lanthanides were constructed using an ordinary least-squares (OLS) regression as well as a weighted least-squares (WLS) regression model for taking into account the heteroscedastic errors. The WLS model was successfully used for a better estimation of the sensitivities and the LODs of the RP-HPLC method than the conventional OLS model. The lanthanide sensitivity obtained from the slope of each calibration curve seems to be better for a lanthanide with an odd-atomic number compared to its neighboring element with an even-atomic number, as if nature is helping us to quantify the concentrations of the less abundant lanthanides. This observation was also confirmed when the LODs computed for all lanthanides were examined. The LODs observed for all lanthanides depicted a clear systematic ''zigzag'' pattern. This is actually the first time that the lanthanide detection limits determined by a HPLC method are shown to mimic the zigzag patterns for the concentration data in geological and cosmological materials. Such a ''zigzag'' pattern should be used as a standard criterion for evaluating the quality of detection limit data.
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1. Introduction

The chemical analysis of lanthanides, also known as rare-earth elements (REEs), is of great importance not only to understand geological processes in Earth sciences [1–3], but also to investigate other tech-nological and scientific fields, such as the develop-ment of superconducting materials, the nuclear in-dustry, and metallurgy [4–6]. Because the lanth-anides are normally present at trace concentration levels in most geological materials and their chemi-cal properties are very similar, their chemical analy-sis has been recognized as a complex analytical task due to either the preparation of representative sam-ples or the presence of matrix interferences [6–8].

Analytical separation methods have been shown to

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be rapid, precise, accurate, and low-cost for the determination of lanthanides in a wide variety of matrices. These methods have commonly included the use of analytical techniques such as high-performance liquid chromatography (HPLC), ion chromatography (IC), and capillary electrophoresis (CE) [2,7–13].

The first chromatographic applications to measure some lanthanides at trace concentrations have been attributed to Cassidy and Elchuck [14]. Such an analytical development was subject of further investigation for analyzing lanthanides in geological matrices by Mazzucotelli et al. [15], who were able to determine fairly high concentrations of only five elements La to Sm (10–160 mg/l). Cassidy [8] suggested the use of reversed-phase (RP) HPLC for nanogram amounts of most lanthanides with a much better precision (0.5–2.0%). Since these early reports, numerous HPLC procedures have been developed for trying to improve the detection and separation of lanthanides, to optimize the analysis time, and to push down the limits of detection (LODs) [6,10,11,16–24].

Most of these chromatographic procedures have employed a gradient elution of the lanthanides using a RP C_{18} analytical separation column with HIBA (α-hydroxyisobutyric acid) as the eluent, sodium n-octanesulfonate as the column modifier and after a post-column reaction (PCR) with some derivatizing or complexing reagents. The detection procedures of lanthanides have normally involved the use of UV–Vis detection at wavelengths that range from 512 to 658 nm. Different complexing reagents, such as Arsenazo III [2,7-bis(o-arsenophenyl)azo-1,8-dihydroxynaphtalene-3,6-disulfonic acid], PAR [(4-(2-pyridylazo)resorcinol], xylenol orange {3,3′-bis[N,N-di(carboxymethyl)aminomethyl] o-cresolsulfonphtalein}, erichrome black T [1-(1-hydroxy-2-naphthylazo)-6-nitro-2-naphtholsulfonate], among others have been proposed for achieving an efficient detection for all lanthanides, and also for suppressing interference problems due to the presence of other cations (e.g., Refs. [2,4,8,10,11,25]).

Absorption spectra measurements have shown that a noteworthy sensitivity enhancement in the absorbance of the lanthanide complexes is normally obtained with Arsenazo III as PCR reagent at wavelengths ranging from 605 to 658 nm. Arsenazo III has been widely recognized as one of the most suitable chromogenic reagents for the determination of lanthanides because it exhibits a high sensitivity to these elements over the pH range of 3–4 [26,27].

In the present work, we have used a RP-HPLC method based on the absorbance measurements produced by the stable complexes, formed by lanthanides with Arsenazo III, to quantify their concentration in synthetic standards. Such a RP-HPLC procedure has been used for improving the sensitivity and selectivity of the lanthanide analysis as well as for lowering the LODs. The objective of this work is to report the results obtained during a comprehensive chromatographic study for the separation and detection of all the lanthanides. Such results have been evaluated in terms of precision (retention times and peak areas), accuracy, and LODs achieved in the chromatographic runs. Customarily, an ordinary least-squares (OLS) regression method is used for constructing calibration curves in chromatographic studies [4,10,28], which is shown to be a statistically erroneous method. Therefore, weighted least-squares (WLS) regression calibration curves for all lanthanides were prepared for taking into account the heteroscedastic errors involved during the chromatographic runs. The WLS method enabled us to compute the straight-line slopes and intercepts with considerably smaller errors than the conventional OLS method, and hence, to obtain a much better estimation of the LODs. The importance of determining correctly the LODs for each individual element of this group is also discussed, since they seem to follow a clear systematic zig-zag pattern.

2. Experimental

2.1. Apparatus

The configuration of the HPLC instrument employed consisted of two high-pressure pumps (Kratos) coupled with a linear gradient programmer (Kratos), a variable-wavelength absorbance detector (set in at 658 nm, Kratos), a C_{18} reversed-phase analytical column (12.5 cm×4.6 mm I.D., Supelco), a pneumatic controller for post-column reagent delivery, and a manual injection valve equipped with sample loops of 50 and 100 μl (Rheodyne, USA).

The HPLC instrument was interfaced to an integrator unit (Spectra-Physics, Model SP4270) for collecting chromatographic data. The PCR reagent (Arsenazo III) was delivered under a helium pressure. The separation column was protected for preventing fouling problems by fixing a guard column (Supelco). The experimental work was performed under gradient eluent flow-rate conditions and at laboratory temperature. The equipment setup at the Max-Planck Institut für Chemie, Mainz, Germany was the same as the one used in earlier studies [8,10,11]. The complete operating conditions and the composition of the HIBA eluent used by the RP-HPLC system during the chromatographic runs are summarized in Table 1.

2.2. Chemical reagents, eluents, post-column reagents, and lanthanide synthetic standards

All chemical and synthetic standard solutions were prepared using deionized water (18 MΩ) that was further purified by a triple distillation system. All chemicals were of reagent suprapure grade. Stock standard solutions containing 1000 mg/l of individual lanthanides were made by acid dissolution of high-purity metal oxides. Working standard solutions of mixed lanthanides were carefully prepared by dilution of stock solutions using PTFE beakers and high-purity acids for avoiding any contamination. All

synthetic lanthanide standards (SPV-1, SPV-2, SPV-3, SPV-4, 81-1A, and 81-B) were prepared at the Max-Planck Institut für Chemie, according to the relative abundance that each lanthanide exhibits in actual geological materials such as basalts, andesites, rhyolites, granites, etc. [10].

The chemical composition pattern of lanthanides (in ng) in these working standard solutions is presented in Table 2. Normally aliquots of 50 or 100 µl were injected into the instrument to obtain chromatograms, except for the SPV-4 standard which was injected as a 75 µl aliquot. Procedure blank solutions containing the same solvent and reagents used with the standards, and subjected to exactly the same sequence of analytical procedures were also prepared and injected daily to obtain blank chromatograms and to check baseline stability.

A stock aqueous solution of HIBA was prepared at a 1 M concentration. This primary solution of HIBA was then used for preparing two working solutions (0.05 and 0.5 M) according to the concentration requirements summarized in Table 1. The post-column reagent (Arsenazo III) was prepared with a mixture of 0.1 mM Arsenazo III and 1 M acetic acid. This solution was stored and protected from the sunlight using amber containers. Prior to the injection into the chromatograph, the standards, eluents, and the PCR reagent were filtered through a 0.2 µm filter-membrane and degassed with helium.

Table 1
Chromatographic operating parameters employed for the determination of lanthanides with a reversed-phase analytical column using UV–Vis detection after PCR

Chromatographic parameter	Description
Mobile phase (eluent) compositions	Eluent A: 0.05 M HIBA+0.01 M sodium n-octanesulfonate Eluent B: 0.5 M HIBA+0.01 M sodium n-octanesulfonate (both adjusted with NH$_4$OH to pH 3.8)
Gradient profile	Linear gradient from 0.05 to 0.5 M HIBA in 16 min (1.0 ml/min)
HPLC analytical column	5 µm C$_{18}$ reversed-phase analytical column (12.5 cm×4.6 mm I.D.)
Composition of PCR	0.1 mM Arsenazo III in 1 M acetic acid
Flow-rate of PCR	0.5 ml/min (regulated by ~1.4 bar of He pressure)
Detection system	UV–Vis at λ=658 nm with a baseline sensitivity of 0.009 AUFS
Sample injection volumes	50, 75 and 100 µl

Table 2
Chemical composition pattern of the lanthanide synthetic standard solutions

Chemical element	Atomic number	Amount (ng)					
		SPV-1 (100 μl)	SPV-2 (100 μl)	SPV-3 (100 μl)	SPV-4 (75 μl)	81-1A (50 μl)	81-B (100 μl)
La	57	148.70	232.00	174.80	240.80	44.27	18.89
Ce	58	298.10	411.00	313.90	432.50	106.90	45.63
Pr	59	46.32	46.09	32.35	44.57	16.36	6.98
Nd	60	181.80	189.20	155.70	214.50	79.80	34.06
(Pm)*	61	–	–	–	–	–	–
Sm	62	42.44	42.21	45.29	48.14	25.00	10.67
Eu	63	11.68	6.92	10.17	10.81	10.18	4.34
Gd	64	44.03	24.71	32.16	34.18	34.41	14.68
Tb	65	93.80	46.58	6.77	7.19	6.33	2.70
Dy	66	45.82	27.81	36.43	22.29	42.32	18.07
Ho	67	10.25	7.18	10.03	6.13	8.83	3.77
Er	68	24.51	17.69	24.74	15.13	27.61	11.78
Tm	69	4.66	2.31	3.79	2.32	4.52	1.93
Yb	70	23.28	11.54	18.59	11.37	28.73	12.26
Lu	71	4.36	2.18	3.55	2.17	4.18	1.79

* (Pm), a radioactive element, is not detectable in Earth's materials. The injection volumes of lanthanides (in μl) are reported below the standard name (in parentheses). Additionally, a blank solution was daily injected to obtain blank chromatograms.

3. Results and discussion

3.1. Conditioning and operation of the HPLC system

Before beginning the chromatographic runs, the HPLC flow system (flow paths, pump, precolumn, and column) was washed by flushing the system with deionized-triply distilled water, at a flow-rate of 1 ml/min for ~30 min, followed by rinsing with 0.05 M HIBA eluent for ~30 min. The HPLC instrument was then set in operation to work with the optimum flow-rate of the working eluent according to the gradient profile indicated in Table 1.

Standard samples (50, 75 or 100 μl) of lanthanides were injected into the mobile phase (eluent), and the HIBA concentration was immediately programmed linearly from 0.05 to 0.5 M over 16 min with a flow-rate of 1.0 ml/min. This flow-rate was maintained constant during the gradient profile. The separated lanthanides were detected after a PCR with Arsenazo III. This PCR reagent was pneumatically delivered to the eluent using a mixing-tee with an average flow-rate of 0.5 ml/min, which was regulated by an average helium pressure of ~1.4 bar. For detecting all lanthanides, the on-line UV–Vis detector was set at a wavelength of 658 nm. The

lanthanide concentration of the standards was quantified using the peak signal of absorbance produced by the metallic-complexes. Such a signal was finally transferred into the integrator unit for converting it in area units of concentration. A fast recovery period was subsequently required for restoring the eluent flow-rate conditions to 0.05 M HIBA. Normally, after a period of 3 min, when the baseline became stable, the system was ready for the next sample injection.

3.2. Separation of the lanthanide group

During each sample injection, the lanthanides were efficiently separated due to differences in the stability of the complexes formed. The reverse elution order was initiated with Lu, the most stable complex formed with HIBA, and terminated with La, the weakest complex produced with HIBA [13]. Fig. 1A and B present two experimental chromatograms showing the complete separation of the lanthanide group using the standard solutions 81-1A and 81-B, respectively. These efficient separations were achieved in less than 15 min. All the lanthanides were readily identified from their retention times because they always exhibited an optimal peak shape without any overlap problem. These output chro-
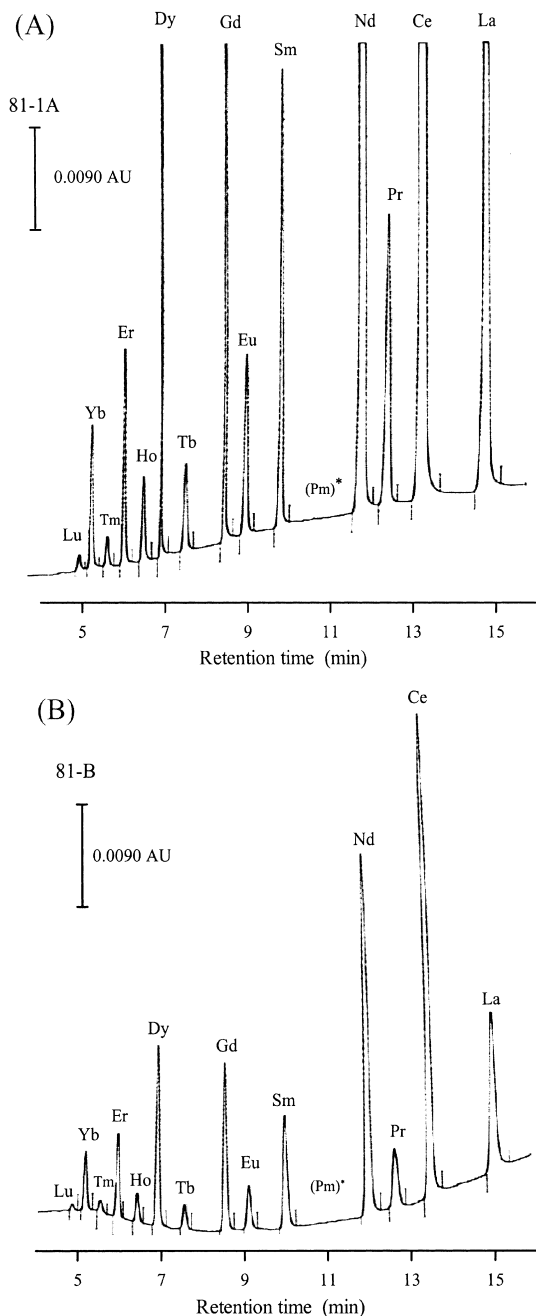
Fig. 1. Separation of the lanthanides in synthetic standard solutions by HPLC with UV–Vis detection (658 nm) after PCR with Arsenazo III using a linear gradient eluent from 0.005 to 0.5 M HIBA (pH 3.8). Other HPLC operating conditions are described in Table 1. Chemical composition patterns of the synthetic standards are summarized in Table 2. (A) A typical chromatogram for synthetic standard solution 81-1A; (B) a typical chromatogram for synthetic standard solution 81-B.

matographic patterns enabled to solve some serious problems commonly encountered in the determination of lanthanides by HPLC, such as those related to separation of Sm–Eu–Gd, the tailing problems of Ho and Yb, and the poor sensitivity exhibited by Tm and Lu peaks [7,24,29]. With respect to the UV–Vis detection at 658 nm, an excellent sensitivity was consistently achieved in all the lanthanide analyses. A good linear response between the peak area and the lanthanide concentration as well as a high signal-to-noise ratio were obtained. Problems related to the baseline noise rarely occurred during the chromatographic runs. When such problems appeared, they were attributed to the Arsenazo III flow-rate, which was normally corrected by regulating the helium pressure of the PCR container at about 1.4 bar.

3.3. Regression methods

Seven concentration levels of each lanthanide including a blank (Table 2) and seven replicates of each concentration level were used for calibration purposes (see Table 3 for peak areas and their standard deviation values). Linearity between the peak area and concentration was observed for all lanthanides (Table 4). Two different regression models were used and compared: an OLS model conventionally used in such studies; and a WLS model proposed as the recommended method to be used in all chromatographic studies.

3.3.1. Ordinary least-squares regression model

The OLS method commonly used for chromatographic calibrations requires several assumptions to be fulfilled [30,31], viz., (1) linearity between y and x variables; (2) x is error-free or less than one-tenth of the error in y; (3) errors in y are normally distributed; (4) homoscedastic error in y (constant variance across the entire response range); and (5) errors associated with different observations are independent.

In our experiments the first assumption seems to be valid because all the calibration curves of the lanthanides showed good squared linear correlation coefficients (r^2) ranging from 0.9393 to 0.9988 (see Table 4), implying statistically significant correlations at the 99% confidence level. The second assumption required a careful evaluation of the errors

Table 3
Heteroscedastic errors propagated during the chromatographic experimental runs

Chemical element	Peak area±s (RSD, %)*						
	SPV-1	SPV-2	SPV-3	SPV-4	81-1A	81-B	Blank
La	1 924 653 ±69 288 (3.6)	3 195 164 ±115 026 (3.6)	2 415 222 ±74 497 (3.1)	3 212 959 ±108 536 (3.4)	544 752 ±33 019 (6.1)	151 775 ±7437 (4.9)	0
Ce	3 264 000 ±68 544 (2.1)	4 384 061 ±157 826 (3.6)	3 412 149 ±34 256 (1.0)	4 498 190 ±140 694 (3.1)	1 264 202 ±5038 (0.4)	496 141 ±15 877 (3.2)	0
Pr	555 122 ±11 658 (2.1)	522 171 ±18 798 (3.6)	341 919 ±12 912 (3.8)	491 886 ±6315 (1.3)	170 746 ±3869 (2.3)	58 121 ±2340 (4.0)	0
Nd	1 768 691 ±37 143 (2.1)	1 936 407 ±69 711 (3.6)	1 561 760 ±20 902 (1.3)	2 135 287 ±68 802 (3.2)	813 672 ±6468 (0.8)	337 240 ±8336 (2.5)	0
(Pm)*	–	–	–	–	–	–	–
Sm	400 805 ±12 826 (3.2)	408 052 ±13 058 (3.2)	410 390 ±12 492 (3.0)	455 971 ±13 678 (3.0)	226 797 ±5244 (2.3)	89 506 ±1877 (2.1)	0
Eu	74 434 ±2155 (2.9)	55 329 ±1771 (3.2)	76 946 ±2151 (2.8)	83 462 ±1331 (1.6)	78 747 ±424 (0.5)	33 952 ±611 (1.8)	0
Gd	257 020 ±8225 (3.2)	126 671 ±4053 (3.2)	215 055 ±643 (0.3)	236 830 ±1285 (0.5)	239 822 ±3623 (1.5)	96 621 ±4388 (4.5)	0
Tb	489 140 ±15 652 (3.2)	303 725 ±9719 (3.2)	37 970 ±183 (0.5)	42 345 ±1903 (4.5)	35 370 ±2252 (6.4)	17 667 ±936 (5.3)	0
Dy	205 424 ±6574 (3.2)	144 320 ±4618 (3.2)	174 916 ±4792 (2.7)	111 597 ±5081 (4.6)	209 435 ±1560 (0.7)	82 606 ±3259 (3.9)	0
Ho	36 448 ±1166 (3.2)	28 777 ±921 (3.2)	36 714 ±736 (2.0)	23 400 ±1159 (5.0)	32 239 ±1370 (4.2)	13 827 ±567 (4.1)	0
Er	70 650 ±2261 (3.2)	57 016 ±1825 (3.2)	72 714 ±1769 (2.4)	46 701 ±1652 (3.5)	82 209 ±1473 (1.8)	35 218 ±1179 (3.3%)	0
Tm	10 646 ±341 (3.2)	5648 ±181 (3.2)	8136 ±83 (1.0)	5645 ±275 (4.9)	11 209 ±789 (7.0)	4517 ±217 (4.8)	0
Yb	41 505 ±1328 (3.2)	23 242 ±744 (3.2)	33 551 ±779 (2.3)	21 828 ±150 (0.7)	53 490 ±1009 (1.9)	23 878 ±603 (2.5)	0
Lu	5903 ±189 (3.2)	2772 ±89 (3.2)	4749 ±222 (4.7)	2930 ±206 (7.0)	6183 ±103 (1.7)	2791 ±142 (5.1)	0

* s=Standard deviation errors; RSD (%)=percentage of relative standard deviation. For blank injections, lanthanide peaks were never observed.

in the concentration (x-variable) as well as in the peak area (y-response variable). The typical errors found in the peak area (y-response variable) measurements of our chromatographic experiments are given in Table 3. Since the lanthanide standard solutions were carefully prepared by weight (using a carefully calibrated sensitive electronic balance with a readout of five decimal fractions), it is, without any

Table 4

Results of the ordinary and weighted least-squares regressions obtained from the individual responses of lanthanides using calibration curves with seven data points*

Chemical element	Atomic number	Quantity injected min–max (ng)	OLS**			WLS ***	
			Intercept ±S.E.	Slope ±S.E.	Linearity (r^2)	Intercept ±S.E.	Slope ±S.E.
La	$\begin{bmatrix} 57 \\ 58 \end{bmatrix}$	0–240.8	−58 778±38 841	$\begin{bmatrix} 13\,796\pm252 \\ 10\,511\pm183 \end{bmatrix}$	0.9983	−4.5±100	$\begin{bmatrix} 12\,878\pm225 \\ 11\,288\pm84 \end{bmatrix}$
Ce		0–432.5	59 610±51 596		0.9984	1.6±100	
Pr	$\begin{bmatrix} 59 \\ 60 \end{bmatrix}$	0–46.3	−15 908±12 986	$\begin{bmatrix} 11\,685\pm394 \\ 9975\pm150 \end{bmatrix}$	0.9943	−24.8±100	$\begin{bmatrix} 10\,839\pm112 \\ 10\,050\pm83 \end{bmatrix}$
Nd		0–214.5	3288±21 732		0.9988	0.4±100	
(Pm)	$\begin{bmatrix} 61 \\ 62 \end{bmatrix}$	–	–	$\begin{bmatrix} - \\ 9515\pm212 \end{bmatrix}$	–	–	$\begin{bmatrix} - \\ 9164\pm34 \end{bmatrix}$
Sm		0–45.3	−6037±7485		0.9975	0.0±100	
Eu	$\begin{bmatrix} 63 \\ 64 \end{bmatrix}$	0–11.7	2751±4987	$\begin{bmatrix} 7089\pm574 \\ 6351\pm606 \end{bmatrix}$	0.9393	19.1±100	$\begin{bmatrix} 7637\pm59 \\ 6668\pm44 \end{bmatrix}$
Gd		0–44.0	350±17 965		0.9564	−5.4±100	
Tb	$\begin{bmatrix} 65 \\ 66 \end{bmatrix}$	0–93.8	6842±10 978	$\begin{bmatrix} 5376\pm276 \\ 4698\pm227 \end{bmatrix}$	0.9870	3.9±100	$\begin{bmatrix} 5654\pm56 \\ 4841\pm61 \end{bmatrix}$
Dy		0–45.8	3562±7092		0.9884	−0.2±100	
Ho	$\begin{bmatrix} 67 \\ 68 \end{bmatrix}$	0–15.1	584±998	$\begin{bmatrix} 3622\pm134 \\ 2928\pm96 \end{bmatrix}$	0.9931	5.9±100	$\begin{bmatrix} 3703\pm53 \\ 2996\pm36 \end{bmatrix}$
Er		0–27.6	1253±1877		0.9946	4.8±100	
Tm	$\begin{bmatrix} 69 \\ 70 \end{bmatrix}$	0–11.4	74±370	$\begin{bmatrix} 2320\pm116 \\ 1804\pm53 \end{bmatrix}$	0.9875	83.7±95	$\begin{bmatrix} 2197\pm38 \\ 1892\pm18 \end{bmatrix}$
Yb		0–28.7	953±918		0.9957	36.0±99	
Lu	[71]	0–4.4	−7.1±212	[1392±71]	0.9870	−103.4±88	[1431±33]

* The least-squares regression results are presented without rounding, as obtained from computer calculations.
** OLS=Ordinary least-squares regression model.
*** WLS=Weighted least-squares regression model.
Note: Brackets [ ] indicate odd-even neighbor lanthanides.

doubt, expected that the concentration (x-variable) errors were indeed much smaller or even negligible compared with those related to the y-variable (peak area). This is true, i.e., the errors in the concentration variable (or the injected amount of lanthanide) are negligible in comparison to the errors in peak area (Table 3), even when the errors (estimated to be around 0.3%) related to the injection of 50 to 100 μl volumes of synthetic lanthanide standards were considered in conjunction with the weighing errors for preparing these standard solutions because the y-response variable errors were much larger, ranging from 0.3 to 7.0% (Table 3). We also checked the third assumption by evaluating the errors in peak areas, which were generally found to have a normal distribution. The errors associated with different observations (assumption 5) can probably be consid-

ered valid because the injections of different standards are independent experiments.

At least one assumption (No. 4) is violated, i.e., homoscedastic error in the y-axis variable, in all chromatographic applications. Note that the intensity values (Table 3) show variable standard deviations (or variances) as well as variable relative standard deviations (RSDs). Violation of this assumption (i.e., heteroscedastic rather than homoscedastic error) means that the OLS is a statistically erroneous method to obtain calibration curves in any chromatographic experiment.

Nevertheless, OLS calibration results are summarized in Table 4. Furthermore, in order to avoid possible outliers in the linear calibration curves (OLS and WLS), an outlier detection algorithm based on suitable statistical tests [32] for detecting

and eliminating outliers from the calibration data was also coupled into the computer code.

It is clearly seen that even if a blank data point (0, 0) is included in the regression curves for all lanthanides, the OLS method gives unusually large intercept values (negative as well as positive values of intercepts), characterized by rather large errors (see the column of ''Intercept±S.E.'' under the OLS columns in Table 4). The corresponding slopes are also characterized by larger errors than the WLS method described below.

3.3.2. Weighted least-squares regression model

Because at least one assumption was violated by the conventional OLS method, a WLS model was used for taking into account the heteroscedastic errors in y-response variable, by assigning to each data point a weight factor inversely proportional to its variance (s^2; Table 3). For constructing the calibration curves for all lanthanides, the statistical theory for a WLS model (reported in some books [30,33]) was codified in a Fortran computer code, which was subsequently applied to our experimental data for estimating the slope and the intercept, including their respective errors. The calibration results predicted by the WLS model were comprehensively compared with those by the OLS model. Significant differences in the slope values by these methods (OLS and WLS) were generally observed. The uncertainties of the slope computed with the WLS model were always significantly smaller than those provided by the OLS model. This is consistent with the suggestion of Miller and Miller [30] that the WLS method generally provides the lowest random errors of the slope, and thus the lowest confidence limits. These results (WLS model) were considered of paramount importance for estimating correctly the LODs because WLS also gives insignificant intercept values (close to zero) for most calibration curves, whereas the OLS method produces much larger intercepts (although with very large errors; Table 4), and, therefore, is not suitable for the estimation of LODs [34]. Furthermore, smaller errors in the slopes of the calibration lines by the WLS model as compared to the OLS model (Table 4) may also mean that more reliable estimates of LODs would be possible by the WLS method rather than the conventional OLS approach.

3.4. Quality parameters

3.4.1. Sensitivity

Systematic differences in the sensitivity for all the lanthanides were clearly observed when the slopes of the calibration curves of the lanthanides were examined (Table 4). Furthermore, these differences are considered statistically significant irrespective of the regression methods used, whether OLS or WLS (see the ''Slope±S.E.'' columns in Table 4). A schematic plot showing the behavior exhibited by the WLS parameters (slope and intercept values) for all lanthanides is presented in Fig. 2. The actual concentration ranges used by the WLS method are given in detail in Table 3.

With respect to the patterns of sensitivities shown by the lanthanide slopes, two points become evident. First, there is a gradual decrease in sensitivity from La to Lu (see Fig. 2). Second, if we consider ''odd–even'' pairs, the sensitivity of an ''odd'' numbered lanthanide is better than the nearest ''even'' numbered lanthanide. For example, for the La–Ce pair, $_{57}$La, an ''odd'' atomic number element, shows a higher sensitivity than $_{58}$Ce, an ''even'' atomic number element. This paired behavior can be observed consistently throughout the lanthanide group (Fig. 2). This systematic ''odd–even'' pattern of lanthanide sensitivities would seem to indicate that nature is helping us to quantify the concentrations of the less abundant elements of this group. Otherwise, it would be somewhat difficult to measure the composition of some odd-numbered lanthanides such as Eu, Tb, Ho, Tm, and Lu in the presence of their neighboring even-numbered elements. It is important to point out that the data for only two lanthanides (La–Ce) presented by Jones et al. [35] are fully consistent with our results. Nevertheless, ours is probably the first study that shows a systematic behavior of sensitivities for all lanthanides in HPLC applications.

3.4.2. Precision

Precision tests based on 32 injections of the lanthanide synthetic standard solution 81-B were performed. Table 5 shows the precision data for retention times and peak areas. The RSDs of retention times were less than 1% for La to Gd, and from 1.2 to 4.2% for the other lanthanides (Table 5).
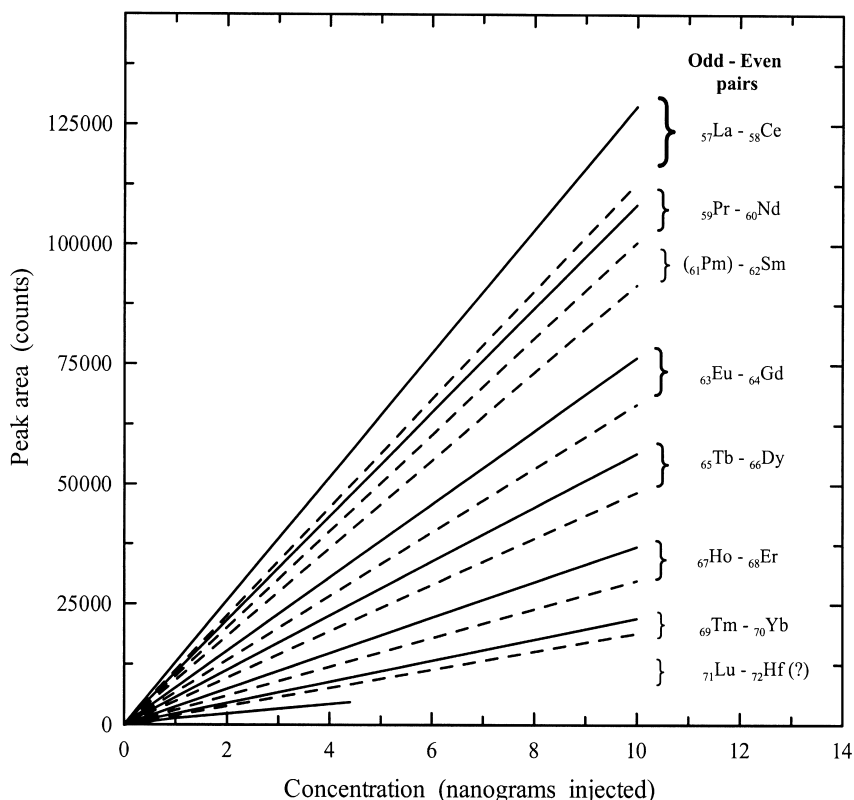
Fig. 2. A schematic plot showing the systematic pattern of the lanthanide sensitivities exhibited during the chromatographic runs. The slopes of the odd-atomic number elements are represented by solid-lines, whereas those corresponding to the even-atomic number elements are indicated by dashed-lines.

Similarly, the RSDs of peak areas ranged from 1.8 to 5.3%. Note the heteroscedastic errors for peak areas obtained for well-controlled precision tests based on a large number of injections ($n=32$).

3.4.3. Accuracy

Accuracy tests were continuously done by the analysis of standard solutions containing the lanthanide group separated from numerous international geochemical reference materials issued from different agencies, such as United States Geological Survey (e.g., GSP-1, BHVO-1, W-1, and RGM-1) and Geological Survey of Japan (e.g., JG-2, JR-1, JA-1, and JGb-1) [3,10,11]. Acceptable accuracy results ranging from 0.5 to about 10% were consistently obtained for the analysis of the lanthanides. A typical chromatogram for JA-1 from Japan is given in Fig. 3, which shows well-resolved lantha-

nide peaks. Also see Y interference on the Dy peak (Y, being a REE, could not be separated from Dy), which did not permit Dy to be quantified in geochemical reference materials [10,11].

3.4.4. Detection limit

The LOD is commonly defined as the analyte concentration which gives an instrumental signal significantly different from a blank or background signal [30,34]. The development or the application of analytical techniques for measuring trace elements requires a reliable method for determining the LODs [36–38]. LODs are frequently estimated from a small number of observations, which gives rise to large uncertainties in estimating correctly their true values [37]. Furthermore, a statistically inappropriate OLS regression method has almost invariably been

Table 5
Reproducibility tests based on 32 injections (100 μl) of the lanthanide synthetic standard solution (81-B) and the limits of detection (LODs) estimated according to the 3s method

Chemical element	Atomic number	Amount injected (ng)	RSD (%)		LOD (ng)[+]
			Retention time	Peak area	
La	57	18.89	0.54	4.9	4.0
Ce	58	45.63	0.55	3.2	5.3
Pr	59	6.98	0.57	4.0	0.7
Nd	60	34.06	0.56	2.5	3.9
(Pm)	61	–	–	–	–
Sm	62	10.67	0.65	2.1	2.0
Eu	63	4.34	0.72	1.8	0.4
Gd	64	14.68	0.79	4.5	2.4
Tb	65	2.70	1.2	5.3	0.8
Dy	66	18.07	1.7	3.9	2.6
Ho	67	3.77	2.1	4.1	0.5
Er	68	11.78	2.6	3.3	1.5
Tm	69	1.93	3.1	4.8	0.6
Yb	70	12.26	3.6	2.5	1.2
Lu	71	1.79	4.2	5.1	0.5

[+] LODs ($n=32$) were estimated by means of the 3s method using t-critical values at 99% confidence level; the slopes of the calibration curves were computed from a weighted least-squares (WLS) regression model.

used for chromatographic calibrations. This practice will result in unreliable estimates of LODs (compare the regression data and their errors for OLS and WLS in Table 4).

In this chromatographic work, the LODs for all lanthanides were determined using the ''3s method'' (s is ''sample'' standard deviation), which has been widely used (e.g., Refs. [34–35,37]). This 3s method estimates, in a simple form, a LOD based on either a blank or a trace-level standard. In the present work, 32 injections ($n=32$) of the lowest-level lanthanide synthetic standard (81-B) were used for estimating the LODs. Blank samples could not be used for these estimates because they never presented any chromatographic responses for the lanthanides. For estimating LODs, standard deviation of the area response for the synthetic standard solution 81B (based on 32 injections) was divided by the slope of the corresponding lanthanide calibration curve (Table 4). This calculation enabled a standard deviation value in concentration units to be estimated, which was finally multiplied by the value of student's t, 2.75 (for the 99% confidence level and for $n-1$ degrees of freedom [30] because the ''3s method'' corresponds roughly to a confidence level of 99%) to compute the LOD of each lanthanide.

The results obtained in this LOD calculation procedure have been included in Table 5. The variations of the LODs are presented in Fig. 4A and compared with Arsenazo III molar absorptivity data for lanthanides [39] in Fig. 4B and element abundances in the solar system [40] in Fig. 4C. An important ''zig-zag'' pattern of the LODs is consistently observed for all lanthanides, according to which the odd atomic number lanthanides depict lower LOD values than the even atomic number neighbor elements. Such an ''odd–even'' effect in LODs is not an exclusive behavior of lanthanides, but has also been observed for metallic elements, such as Co, Ni, Cu, and Zn [41]. In fact, it is also convenient to mention that the same kind of LOD patterns has also been observed in other analytical techniques such as inductively coupled plasma (ICP) mass spectrometry, ICP-atomic emission spectrometry (AES), and instrumental neutron activation analysis (INAA) [3,42,43].

A reasonable analytical or physico-chemical cause should be found for explaining this systematic behavior of the LODs for the lanthanides. Some authors have mentioned that LODs in HPLC methods depend critically upon the eluent type, the flowrates of the post-column reagent or the pump pulsations [44]. However, since in our chromatographic experiments such parameters were carefully con-
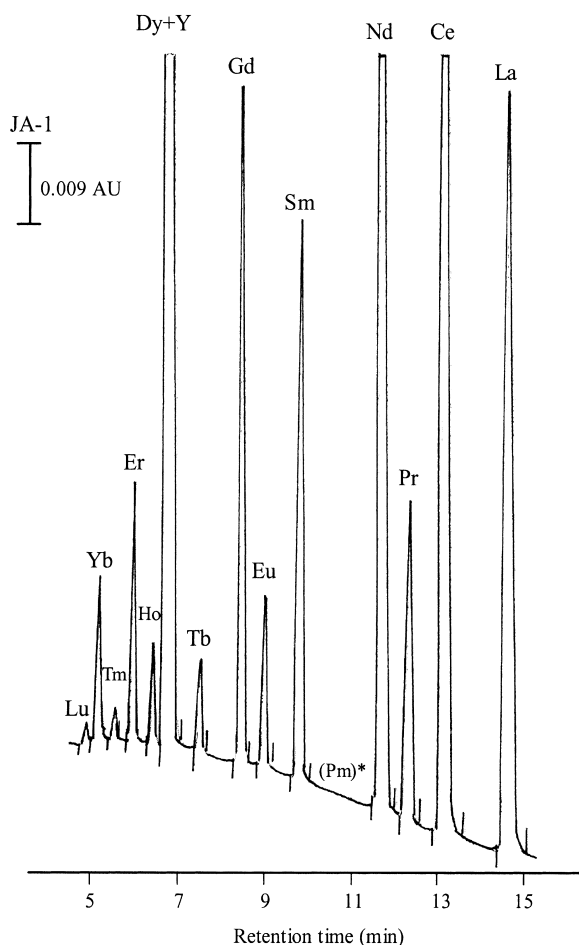
Fig. 3. A typical chromatogram for lanthanide group separated from international geochemical reference material, andesite JA-2 from Geological Survey of Japan (group separation was carried out using cation-exchange columns [10,11]). Note Y interference on the Dy peak.
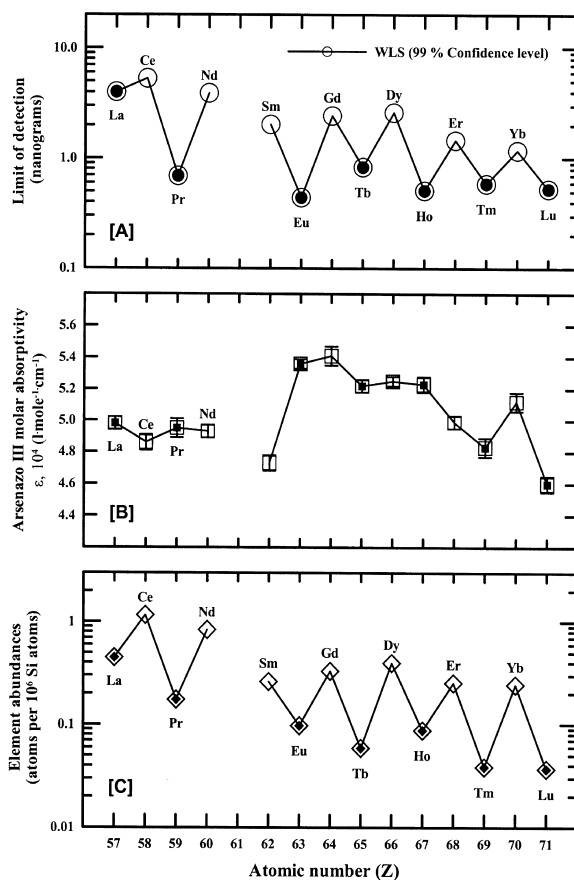

Fig. 4. Patterns for lanthanides. (A) Limits of detection (LODs) computed for all lanthanides by RP-HPLC (see Table 5 for more details); (B) Arsenazo III molar absorptivities for lanthanides; (C) cosmic abundances of lanthanides.

trolled, it is unlikely that they could explain such ''zig-zag'' patterns of LOD variations. The variations in molar absorptivity data for lanthanides do not fully match with the LOD values of lanthanides (Fig. 4B), so this parameter cannot explain the variations of LODs (Fig. 4A). This leads us to suggest that more experimental studies are required to determine more reliable molar absorptivity data. On the other hand, note that lanthanide concentrations in the solar system (Fig. 4C) and in most geological and cosmological materials [3], do match the pattern of LOD values (Fig. 4A).

We propose that the zig-zag pattern in LOD values of the lanthanides should be used as a standard analytical criterion for their evaluation. Furthermore, the estimation of these LODs should be based on at least 30 or more individual measurements [45]. Finally, we suggest that sensitivity data and all WLS parameters should also be reported routinely.

Carefully measured LOD values for lanthanides by HPLC, although being essential parameters, have not been generally reported in most of the chromatographic literature. Instead, they are usually reported as a range or as a single value adopted for most of these elements. This practice should be avoided in favor of a careful report of LODs in all future experiments.

4. Conclusions

WLS regression was successfully applied for HPLC calibrations to determine lanthanide concentrations in synthetic standards. The WLS is the recommended method for all chromatographic studies as compared to the conventional OLS method. The sensitivities for the lanthanides by HPLC show a gradual decrease from La to Lu. Furthermore, the sensitivities of ''odd'' numbered lanthanides are better than the nearest ''even'' numbered lanthanides. This is the first study that shows a systematic behavior of sensitivities for all lanthanides in HPLC applications. The present careful study shows a systematic ''zig-zag'' pattern of LOD values for the lanthanides. Furthermore, sensitivity data and all WLS parameters should also be reported routinely.

References

[1] P. Henderson, Rare Earth Element Geochemistry, Elsevier, Amsterdam, 1984.
[2] M.R. Buchmeiser, G. Seeber, R. Tessadri, Anal. Chem. 72 (2000) 2595.
[3] S.P. Verma, E. Santoyo, F. Velasco-Tapia, Int. Geol. Rev. 44 (2002) 287.
[4] R.T. Gettar, E.A. Gautier, R.E. Servant, D.A. Batistoni, J. Chromatogr. A 855 (1999) 111.
[5] T.P. Rao, V.M. Biju, Crit. Rev. Anal. Chem. 30 (2000) 179.
[6] M.R. Buchmeiser, Rev. Anal. Chem. 20 (2001) 161.
[7] S.P. Verma, R. Garcia, E. Santoyo, A. Aparicio, J. Chromatogr. A 884 (2000) 317.
[8] R.M. Cassidy, Chem. Geol. 67 (1988) 185.
[9] P.R. Haddad, P.E. Jackson, Ion Chromatography: Principles and Applications, Journal of Chromatography Library, Vol. 46, Elsevier, Amsterdam, 1990.
[10] S.P. Verma, Lanthan. Actin. Res. 3 (1991) 237.
[11] S.P. Verma, Geostand. Newslett. 15 (1991) 129.
[12] P.R. Haddad, J. Chromatogr. A 770 (1997) 281.
[13] C. Sarzanini, J. Chromatogr. A 850 (1999) 213.
[14] R.M. Cassidy, S. Elchuck, J. Liq. Chromatogr. 4 (1981) 379.
[15] A. Mazzucotelli, A. Dadone, R. Frache, F. Baffi, J. Chromatogr. 349 (1985) 137.
[16] A.P. Le Roex, R.T. Watkins, Chem. Geol. 88 (1990) 151.
[17] R.T. Watkins, A.P. Le Roex, Geochem. J. 26 (1992) 241.
[18] R. Kuroda, T. Wada, Y. Kokubo, K. Oguma, Talanta 40 (1993) 237.
[19] R.T. Watkins, M.K. Ridley, M.A.B. Pougnet, J.P. Willis, Chem. Geol. 121 (1995) 273.
[20] M.C. Bruzzoniti, E. Mentasti, C. Sarzanini, M. Braglia, G. Cocito, J. Kraus, Anal. Chim. Acta 322 (1996) 49.
[21] H. Lu, S. Mou, Y. Yan, F. Liu, K. Li, S. Tong, J.M. Raviello, Talanta 45 (1997) 119.
[22] P.N. Nesterenko, P.N. Jones, J. Chromatogr. A 804 (1998) 223.
[23] A. Tsuyoshi, K. Akiba, Anal. Sci. 16 (2000) 843.
[24] N.R. Raut, P.G. Jaison, S.K. Aggarwal, J. Chromatogr. A 959 (2002) 163.
[25] A.M.G. Figueiredo, N.M.P. de Moraes, H.M. Shihomatsu, J. Radioanal. Nucl. Chem. 244 (1999) 615.
[26] S.B. Savvin, Talanta 8 (1961) 673.
[27] H. Rohwer, E. Hosten, Anal. Chim. Acta 339 (1997) 271.
[28] J. Weiss, Ion Chromatography, 2nd ed., VCH, Weinheim, 1995.
[29] K.L. Nash, M.P. Jensen, Sep. Sci. Technol. 36 (2001) 1257.
[30] J.N. Miller, J.C. Miller, Statistics and Chemometrics for Analytical Chemistry, 4th ed., Prentice Hall, Edinburgh, 2000.
[31] K. Baumann, H. Wätzig, Proc. Control. Qual. 10 (1997) 59.
[32] V. Barnett, T. Lewis, Outliers in Statistical Data, 3rd ed., Wiley, Chichester, 1994.
[33] P.R. Bevington, Data Reduction and Error Analysis for Physical Sciences, 1st ed., McGraw-Hill, New York, 1969.
[34] G.L. Long, J.D. Winefordner, Anal. Chem. 55 (1983) 713A.
[35] P. Jones, T. Williams, L. Ebdon, Anal. Chim. Acta 237 (1990) 291.
[36] L.E. Vanatta, D.E. Coleman, J. Chromatogr. A 770 (1997) 105.
[37] M. Thompson, Analyst 123 (1998) 405.
[38] L.A. Currie, Anal. Chim. Acta 391 (1999) 127.
[39] D.B. Gladilovich, V. Kubáň, L. Sommer, Talanta 35 (1988) 259.
[40] K.H. Wedepohl, Geochemistry, Holt, Rinehart, and Winston, New York, 1971.
[41] E. Santoyo, S. Santoyo-Gutiérrez, S.P. Verma, J. Chromatogr. A 884 (2000) 229.
[42] S.P. Verma, E. Santoyo, in: Proceedings of the Silver Jubilee ISMAS Symposium, Goa, 2003, p. 471.
[43] S.P. Verma, E. Santoyo, in: 2003 European Winter Conference on Plasma Spectrochemistry, Garmisch-Partenkirchen, 2003, p. 395, Abstract.
[44] E.A. Gautier, R.T. Gettar, R.E. Servant, D.A. Batistoni, J. Chromatogr. 770 (1997) 75.
[45] S.P. Verma, E. Santoyo, in: Proceedings of the Silver Jubilee ISMAS Symposium, Goa, 2003, p. 511.