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# Detailed study on simultaneous separation of rare earth elements by capillary electrophoresis

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**Abstract**

Separation of all rare earth elements (REEs) by capillary zone electrophoresis was investigated in a system of α-hydroxyisobutyric acid (HIBA) as a main complex reagent and acetic acid (HAc) as an assistant complex reagent. In the combined system, ligand $Ac^-$ plays an important role in improving separation of Eu and Gd, and Y and Dy. The calculated ratio of $Ac^-$ to $HIB^-$ concentrations was compared and demonstrated that Eu and Gd, and Y and Dy tend to be separated at lower, and higher ratio of the two free ligands, respectively. An operational buffer system was developed for a complete separation of all REE ions.


*Keywords:* Rare earth elements; Europium; Gadolinium; Yttrium; Dysprosium; α-Hydroxyisobutyric acid; Acetic acid; Capillary zone electrophoresis

## 1. Introduction

As a promising analytical technique for simultaneous separation and determination of analytes, the appearance of capillary electrophoresis (CE) immediately attracted analysts to apply it to analytical chemistry of rare earth elements [REEs: elements of lanthanide group (La–Lu), yttrium and scandium] [1–4]. The reason is that separation and determination of REE analytes are especially challenging due to the similarity in chemical characteristics arising from their equal charge and almost similar ionic radii [5]. In the past decade, application of CE technique to REE analytical chemistry was mainly focused on development of methodology for simultaneous separation and sensitive determination of lanthanides [6–10]. For on-column separation of REE ions, α-hydroxyisobutyric acid (HIBA) is one of the most important reagents [1–3,11]. However, only dependent on the labile complexation of HIBA with lanthanides, effective separation of Eu and Gd has not been achieved by capillary zone electrophoresis (CZE), which is the most popular CE technique. In REE analysis by CE technique, electrophoretic mobility of Y is very close to that of Dy so effective separation of Y and Dy is practically important [11] due to the simultaneous occurrence of Y with lanthanides in natural samples [12]. So far, only an investigation of Y and Dy involved to REE separation by CZE has been reported by using a HIBA–malonic acid system [13]. Detailed study on Y and Dy separation by CZE technique is still in urgent need.

As a separation parameter of running buffer, pH plays a key role in effective separation of analytes. Acetic acid is often selected for adjustment of buffer pH [1,2,11,14,15], and this means acetic acid not only is a pure pH controller but also participates to separate REE ions by complexing reaction [2]. Unfortunately, its effect on separation of REE analytes has not been paid enough attention.

In this study, acetic acid has been employed not only for controlling pH but also as a complex reagent as important as HIBA. Therefore, the concentrations of HAc and HIBA were combined and then carried out for REE sepa-

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ration. Objectives of this work are to systematically investigate the parameters for optimizing separation of Eu and Gd, and Y and Dy, respectively, and to achieve a complete separation of all rare earth elements (scandium, yttrium and 14 lanthanides).

## 2. Experimental

### 2.1. Instrumentation

A P/ACE MDQ capillary electrophoresis instrumentation (Beckman Coulter, CA, USA) equipped with a variable-wavelength UV detection system at the "Centro de Investigacion en Energia" was used in this study. The UV detection was set at a wavelength of 214 nm. The analysis voltage was 12 kV. Sample was introduced at anode side by voltage mode (4 kV, 40 s). Separations were carried out using a fused-silica capillary with 50 cm (effective length: 40 cm) × 75 μm i.d. at 25 °C in a temperature-controlled room. The capillary was flushed with 1 M NaOH, water and running buffer each for 15 min at the beginning. Between runs, 3 min for washing with the buffer was applied. The CE instrument control and data processing were manipulated using Windows NT 4.0 operation system and 32 Karat software.

### 2.2. Reagents

Water was de-ionized with a Milli-Q system (Millipore Mexico, DF, Mexico) to a resistivity of 18 MΩ $cm^{-1}$ and was employed throughout for experimental preparation. Creatinine, α-hydroxyisobutyric acid (HIBA) and acetic acid (HAc) were of analytical reagent grade.

### 2.3. Standards

Mono-elemental stock solutions of 1000 μg/mL in 3% $HNO_3$, respectively, prepared from oxides of $La_2O_3$, $CeO_2$, $Pr_6O_{11}$, $Nd_2O_3$, $Sm_2O_3$, $Eu_2O_3$, $Gd_2O_3$, $Tb_4O_7$, $Y_2O_3$, $Dy_2O_3$, $Ho_2O_3$, $Er_2O_3$, $Tm_2O_3$, $Yb_2O_3$, $Lu_2O_3$ and $Sc_2O_3$ (Inorganic Ventures, NJ, USA) were used. A REE synthetic standard solution of 100 μg/mL for each was prepared using these stock solutions. Working standard solutions, 10 μg/mL, were diluted from the synthetic standard.

## 3. Results and discussion

### 3.1. Effect of creatinine concentration

Creatitine is commonly accepted as ion-probe for indirect detection of cation analytes, and its concentration in running buffer generally occurs at 30 mM [3,16]. Significant influence of ion-probe concentration and absorptivity on analyte detection limit and thereby sensitivity can be estimated in terms of the following equation [17]:

$$C_{\mathrm{LOD}} = \frac{C_{\mathrm{p}}}{RD_{\mathrm{r}}} \quad (1)$$

where $C_{\mathrm{LOD}}$ is the concentration limit of detection, $C_{\mathrm{p}}$ the probe concentration, $R$ the transfer ratio (the number of moles of the probe displaced by 1 mol of analyte) and $D_{\mathrm{r}}$ the dynamic reserve (the ratio of background absorbance to noise). The equation clearly shows higher probe concentration will increase the detection limit. Meanwhile, the dynamic reserve will also become higher and accordingly the detection limit will be decreased. Therefore, the detection limit does not simply correlate with the increase or decrease in the probe concentration. As a result, each of ion-probes should correspond to a suitable concentration, which enables analytes with lower detection limits. In order to obtain such a concentration, a series of creatinine concentrations from 10 mM with increment of 5 mM were experimented. The electropherograms are presented in Fig. 1 for creatinine concentrations of 10, 15 and 20 mM, respectively, and clearly shows the concentration of creatinine not only influences detection sensitivity but also plays some part role in separation of REE analytes. With increase in creatinine concentration, migration times of REE ions become longer and this is attributed to pH increasing. Because of its weakly basic property, the higher the total concentration ($C_{\mathrm{t}}$) of creatinine and the higher pH of the running buffer. Effect of creatinine on separation parameters was estimated based on pH measurements and an assumption of ion strength equal to zero. As shown in Table 1, with increasing pH the free ligand concentrations ($C_{\mathrm{ion}}$) of both $HIB^-$ and $Ac^-$ increase, which are caused by increasing dissociation. Consequently, the degree of complexation of REE ions increases too. With increasing number of ligands in REE complexes the average degree of complexation increases, and both gross charge and electrophoretic mobility decrease. This should lead to a longer migration time.

Using 10 mM creatinine, Sc cannot be observed, baseline separation of Tm, Yb and Lu cannot be achieved, peak shapes of light REE ions are obvious asymmetry due to a severe front peak, and some part separation of both Eu and Gd, and Y and Dy can be observed (Fig. 1A). Using 15 mM creatinine, effective separation of all REE ions is achieved but the peak shape of La occurs with a little front peak (Fig. 1B). Using 20 mM creatinine, although all REE analytes are successfully separated, instable baseline is very striking and the detected signal is significantly decreased (Fig. 1C). Experiments on creatinine concentration demonstrate that 17 mM creatinine in running buffer enables all of rare earth elements to be separated with good shapes and a smooth baseline.

### 3.2. Effect of HIBA concentration

As shown in Fig. 2, with increase in HIBA concentration, migration times of REE ions become longer. This should be attributed to the increase in $HIB^-$ concentration, which covaries with HIBA concentration if pH is constant. As shown

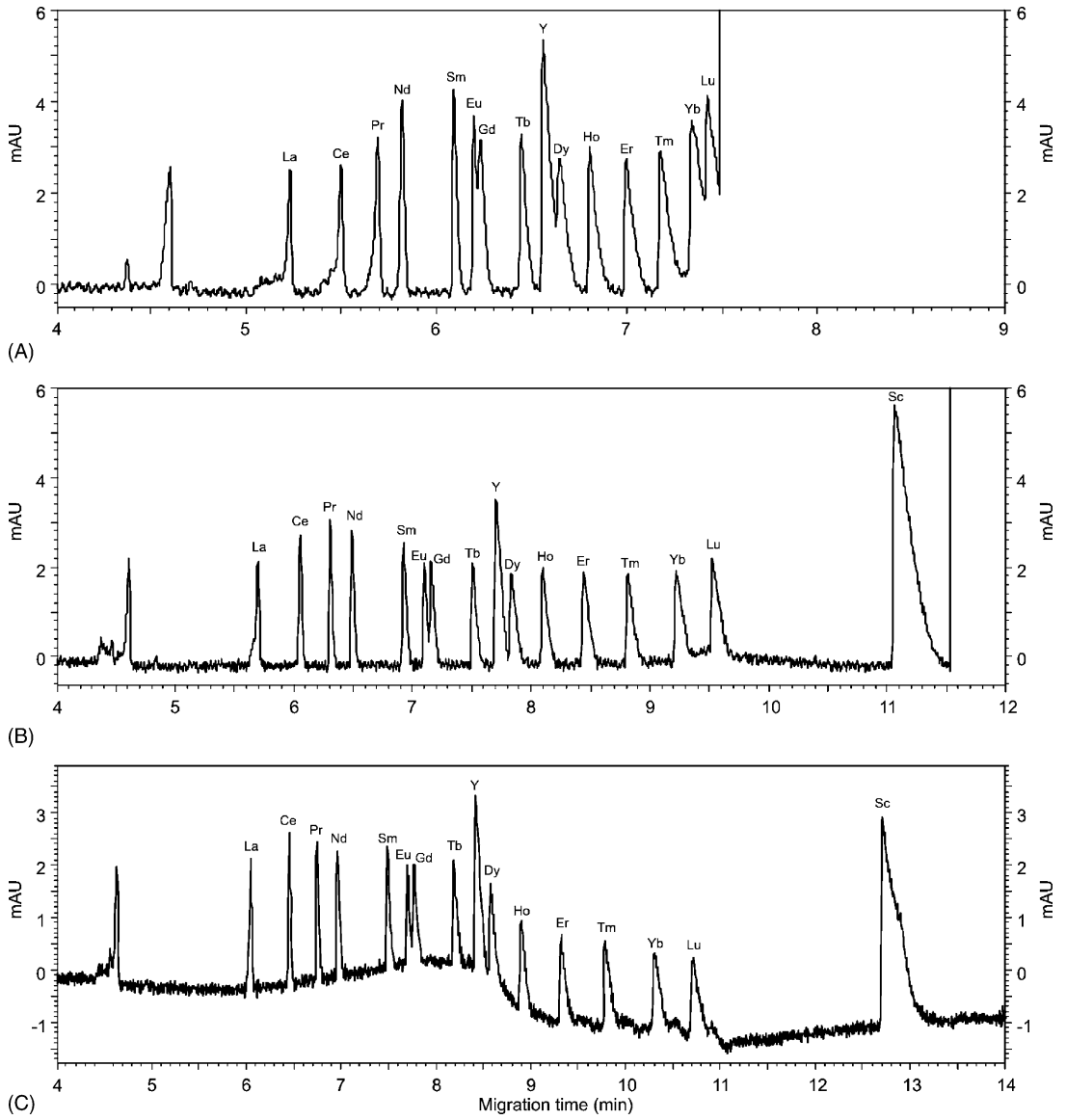


Fig. 1. Separation of rare earth elements with increase in creatinine concentrations: (A) 10 mM; (B) 15 mM; (C) 20 mM. All running buffers contain 7.8 mM HIBA and 175 mM HAc. Injection: 4 kV, 40 s. Applied voltage: 12 kV.

Table 1
Separation parameters of running buffer at different creatinine concentrations

| Creatinine ($pK_a$ = 4.828) | | HIBA ($pK_a$ = 3.971) | | HAc ($pK_a$ = 4.756) | | $[Ac^-]/[HIB^-]$ | pH |
|---|---|---|---|---|---|---|---|
| $C_t$ (mM) | $C_{ion}$ (mM) | $C_t$ (mM) | $C_{ion}$ (mM) | $C_t$ (mM) | $C_{ion}$ (mM) | | |
| 10 | 9.70 | 7.8 | 1.71 | 175 | 6.19 | 3.62 | 3.32 |
| 15 | 14.35 | 7.8 | 1.90 | 175 | 8.80 | 4.63 | 3.48 |
| 20 | 18.88 | 7.8 | 2.33 | 175 | 11.42 | 4.90 | 3.60 |

Table 2
Separation parameters of running buffer at different HIBA concentrations

| Creatinine ($pK_a$ = 4.828) | | HIBA ($pK_a$ = 3.971) | | HAc ($pK_a$ = 4.756) | | $[Ac^-]/[HIB^-]$ | pH |
|---|---|---|---|---|---|---|---|
| $C_t$ (mM) | $C_{ion}$ (mM) | $C_t$ (mM) | $C_{ion}$ (mM) | $C_t$ (mM) | $C_{ion}$ (mM) | | |
| 17 | 16.07 | 5 | 1.47 | 175 | 11.18 | 7.60 | 3.59 |
| 17 | 16.13 | 7 | 1.96 | 175 | 10.48 | 5.35 | 3.56 |
| 17 | 16.16 | 9 | 2.43 | 175 | 10.03 | 4.13 | 3.54 |

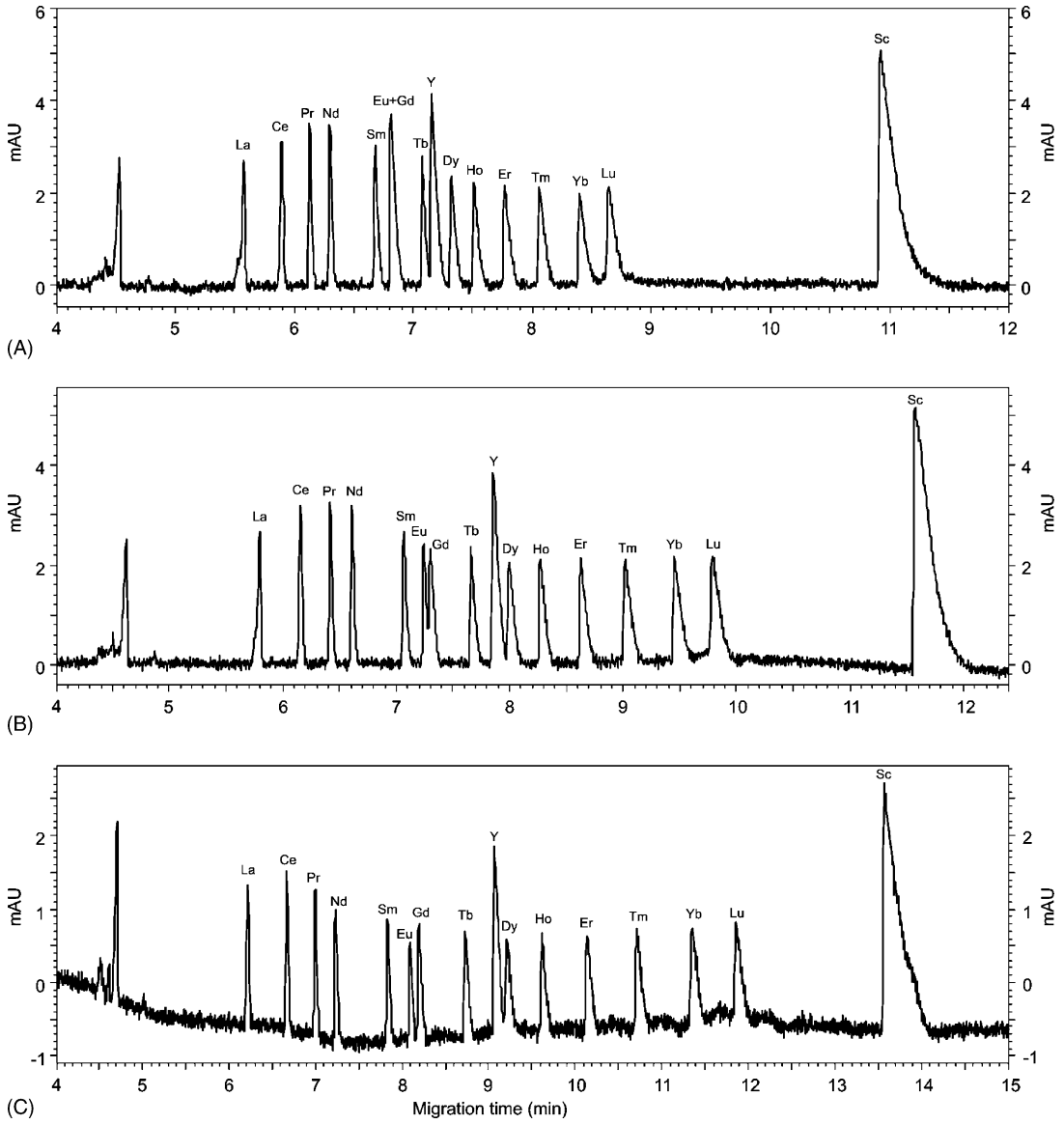


Fig. 2. Separation of rare earth elements with increase in HIBA concentrations: (A) 5 mM; (B) 7 mM; (C) 9 mM. All running buffers contain 17 mM creatinine and 175 mM HAc. Other conditions as in Fig. 1.

in Table 2, the measured pH values are basically constant. Accordingly, free $Ac^-$ concentration, in effect, would change insignificantly. As a result of increase in complexing degree of REE ions with $HIB^-$, number of the ligand in REE complex increases. Consequently, the average degree of complexation increases, and both gross charge and electrophoretic mobility decrease. This should result in a longer migration time.

Since free ligands of $HIB^-$ and $Ac^-$ coexist in the HIBA–HAc system, a competition of complexation of REE ions with $HIB^-$ and $Ac^-$ can be expected. Ion concentration ratio of $Ac^-$ to $HIB^-$ is most likely a major factor for improving separation of Eu and Gd, and Y and Dy. Therefore, their concentration ratio is also involved to running buffer parameters for understanding the separability of Eu and Gd, and Y and Dy. For 5 mM HIBA with a ratio of 7.6 for $Ac^-$ to $HIB^-$, good separation of individuals except for Eu and Gd is obtained and the migration time of Y is closer to Tb than to Dy (Fig. 2A). For 7 mM HIBA with a ratio of 5.4 for $Ac^-$ to $HIB^-$, separation of Eu and Gd is obviously improved relative to 5 mM HIBA and the migration time of Y is closer to Dy than to Tb (Fig. 2B). For 9 mM HIBA with a ratio of 4.1 for $Ac^-$ to $HIB^-$, although effective separation of both Eu and Gd, and Y and Dy is achieved, baseline is not smooth and observed signals are lower than those observed from lower HIBA concentrations (Fig. 2C).

### 3.3. *Effect of HAc concentration*

As shown in Fig. 3, with increase in HAc concentration, there is no significant increase in migration times of REE ions except for Sc. However, observed sensitivity, particularly for light REE ions, is decreased with increasing HAc

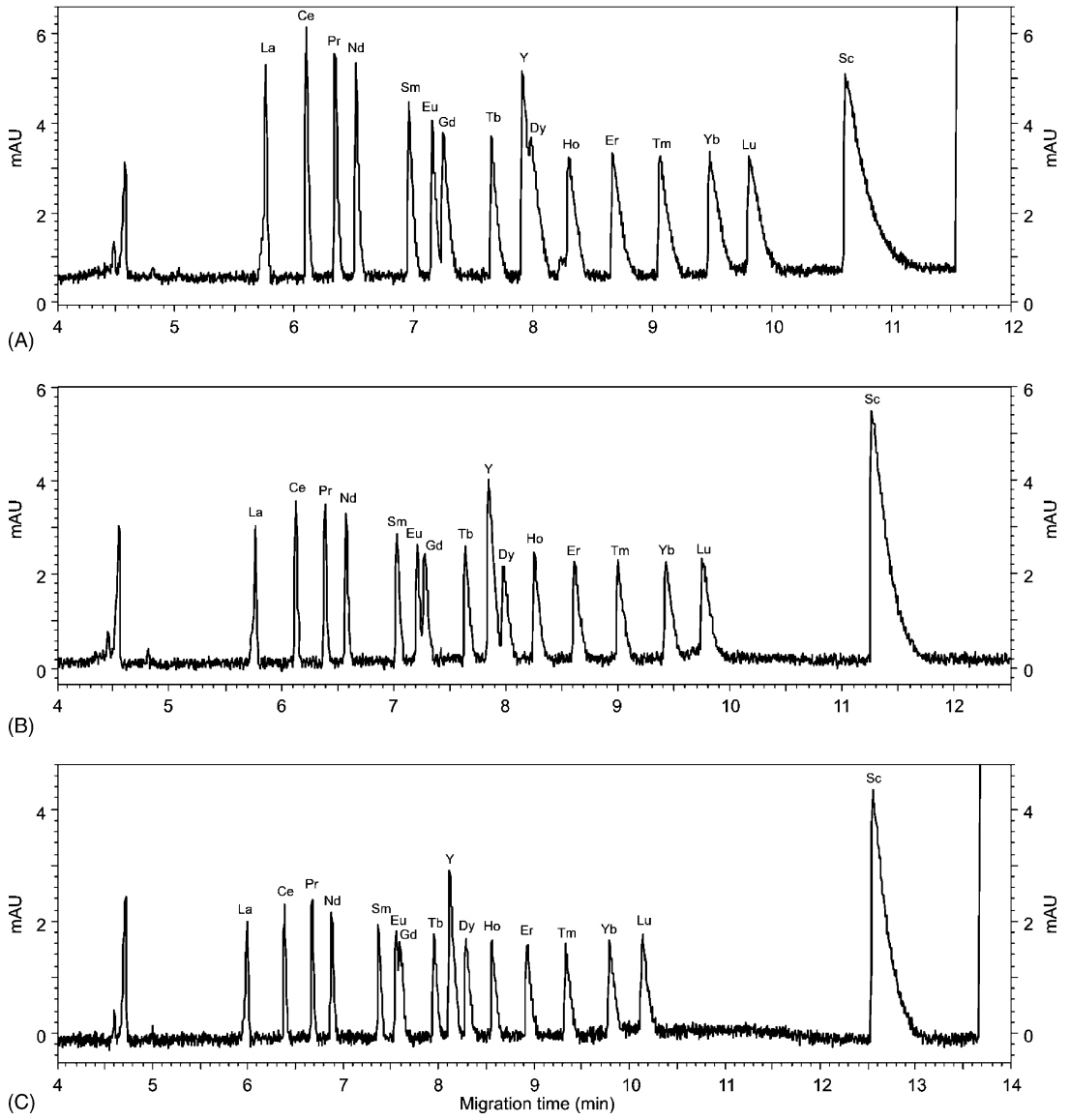


Fig. 3. Separation of rare earth elements with increase in HAc concentrations: (A) 122.5 mM; (B) 175 mM; (C) 245 mM. All running buffers contain 17 mM creatinine and 7.8 mM HIBA. Other conditions as in Fig. 1.

Table 3
Separation parameters of running buffer at different HAc concentrations

| Creatinine ($pK_a$ = 4.828) | | HIBA ($pK_a$ = 3.971) | | HAc ($pK_a$ = 4.756) | | $[Ac^-]/[HIB^-]$ | pH |
|---|---|---|---|---|---|---|---|
| $C_t$ (mM) | $C_{ion}$ (mM) | $C_t$ (mM) | $C_{ion}$ (mM) | $C_t$ (mM) | $C_{ion}$ (mM) | | |
| 17 | 15.84 | 7.8 | 2.68 | 122.5 | 9.69 | 3.62 | 3.69 |
| 17 | 16.13 | 7.8 | 2.18 | 175 | 10.48 | 4.81 | 3.56 |
| 17 | 16.36 | 7.8 | 1.71 | 245 | 10.80 | 6.32 | 3.42 |

concentration. In relation to the separations in Fig. 3, separation parameters are calculated and presented in Table 3. Despite the large difference in HAc concentration used from 122.5 to 245 mM, there is no significant variation in the effective concentration of $Ac^-$. The reason is attributed to the reverse effects of HAc concentration and pH value on $Ac^-$ concentration, respectively. However, the change in HAc concentration affects the buffer pH, which is closely related to the dissociation of HIBA. With increasing HAc concentration the buffer pH and the ion concentration of $HIB^-$ decrease. Consequently, number of $HIB^-$ in REE complexes and the average degree of complexation decrease. As expected, both gross charge and electrophoretic mobility increase, and this would cause a shorter migration time. In effect, Fig. 3 shows a slightly longer migration time. As shown in Table 3, despite by a small amplitude the ion concentration of $Ac^-$ increases

Table 4
Stability constants of Eu, Gd, Y and Dy complexes with HIBA and HAc [21]

| | Eu | Gd | Y | Dy |
|---|---|---|---|---|
| HIBA | $\log K1 = 3.09$ | $\log K1 = 3.08$ | $\log K1 = 3.20$ | $\log K1 = 3.27$ |
| | $\log K2 = 5.54$ | $\log K2 = 5.51$ | $\log K2 = 5.79$ | $\log K2 = 5.90$ |
| HAc | $\log K1 = 2.13$ | $\log K1 = 2.02$ | $\log K1 = 1.68$ | $\log K1 = 1.85$ |
| | $\log K2 = 3.64$ | $\log K2 = 3.47$ | $\log K2 = 3.17$ | $\log K2 = 3.16$ |

with increasing HAc concentration. Accordingly the increase in the degree of complexation of REE ions with $Ac^-$ leads to the increase in number of $Ac^-$ in REE complexes and the average degree of complexation. A longer migration time was expected as a result of the decrease in both gross charge and electrophoretic mobility. Therefore, the slightly longer migration time reflects a combined effect of $HIB^-$ and $Ac^-$ complexes on electrophoretic mobilities of REE ions.

For 122.5 mM HAc with a ratio of 3.6 for $Ac^-$ to $HIB^-$, good separation of Eu and Gd is obtained but separation of Y and Dy cannot be achieved (Fig. 3A). Using 175 mM HAc with a ratio of 4.8 for $Ac^-$ to $HIB^-$, sufficient separation of Eu and Gd, and Y and Dy was obtained at the same time. For 245 mM HAc with a ratio of 6.3 for $Ac^-$ to $HIB^-$, successful separation of Y and Dy is achieved but separation of Eu and Gd becomes impossible.

### 3.4. Optimized separation

The above experiments reveal the separability of Eu and Gd, and Y and Dy in HIBA–HAc system, respectively: Eu and Gd tend to be separated at lower concentration ratio of $Ac^-$ to $HIB^-$, but Y and Dy tend to be separated at higher concentration ratio of $Ac^-$ to $HIB^-$. The separation behavior of Eu and Gd is opposite to that of Y and Dy, and this reveals the reason that good separation of Eu and Gd, and impossible separation of Y and Dy at the same time are generally observed, and vice versa [11]. After optimized, an operational running buffer was compromised for simultaneously separating Eu and Gd, and Y and Dy: 17 mM creatinine, 7.8 mM HIBA and 175 mM HAc (Fig. 3B).

Such a complete separation of all 16 REE ions (Fig. 3B) further proves some advantages of a multi-complex system, which can improve REE separation. Such a combined system has been successfully applied to separating lanthanides, for example HIBA–HAc system [16], cupferron–HIBA system [18], arsenazo III–citrate system [19] and pyridine-2-carboxylic acid–HIBA–formic acid system [20]. By using a HIBA–malonic acid system, good separation of Y and Dy without loss of separation of Eu and Gd was obtained by Mao et al. [13]. They stated this separation should be attributable to that the combined effects of complex species and possible cross complex species enable their electrophoretic mobilities to be differentiated from each other. Similarly, complex species of $[REE(HIB)_n]^{3-n}$ and $[REE(Ac)_n]^{3-n}$, and cross complex specie of $[REE(HIB)(Ac)]^+$ coexist in the HIBA–HAc system [2]. Despite the known coordination number up to three for REE complexes with both $HIB^-$ and $Ac^-$ [2,21], those complex stability constants are compared and shown in Table 4 for Eu, Gd, Y and Dy complexes with coordination number of 1 and 2 because the electrophoretic mobility of the uncharged complexes of $REE(HIB)_3$ and $REE(Ac)_3$ is zero. The stability constants between Eu and Gd complexes with $HIB^-$ are so similar that Eu and Gd complex species co-migrate in the system containing only HIBA as a complex-forming reagent. In contrast, the difference in stability constants between Eu and Gd complexes with $Ac^-$ is larger than that between Eu and Gd complexes with $HIB^-$. By addition of HAc to HIBA system, complex species of $[REE(HIB)_n]^{3-n}$, $[REE(Ac)_n]^{3-n}$ and $[REE(HIB)(Ac)]^+$ form and their combined effects enable the electrophoretic mobility of Eu to be differentiated from that of Gd. As shown in Table 4, the stability constant difference between Y and Dy complexes with $HIB^-$ is larger than that between Eu and Gd complexes with $HIB^-$. For the Y and Dy complexes with $Ac^-$, there is some difference only for the stability constants of $[Y(Ac)]^{2+}$ and $[Dy(Ac)]^{2+}$, different from Eu and Gd complexes with $Ac^-$. This comparison indicates the complexes of Eu and Gd with $HIB^-$ and $Ac^-$ are fairly different from those of Y and Dy with $HIB^-$ and $Ac^-$. It should be the reason that the different characteristics of these complexes enable the separability of Eu and Gd different from that of Y and Dy in the HIBA–HAc system.

Using the operational buffer system, multi-measurement of the migration times of individual REE analytes was conducted, and the reproducibility (R.S.D. (%)) is presented in Table 5. For the elements of lanthanide group, the reproducibility becomes worse with the increase in atomic number but they have a good covariation ($r^2 = 0.9889$ correlation coefficient). Following is their relationship:

$$Y = 0.1045 \times X - 4.8031 \quad (2)$$

where $Y$ is reproducibility (R.S.D. (%)) of migration times and $X$ atomic number of lanthanides.

The correlation coefficient for heavy elements of lanthanide group (Tb–Lu) is $r^2 = 0.9978$, which is higher than the correlation coefficient of $r^2 = 0.9830$ for light elements of lanthanide group (La–Sm).

Table 5
Migration times and reproducibility of rare earth elements

| Element | La | Ce | Pr | Nd | Sm | Eu | Gd | Tb | Y | Dy | Ho | Er | Tm | Yb | Lu | Sc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Atomic number | 57 | 58 | 59 | 60 | 62 | 63 | 64 | 65 | 39 | 66 | 67 | 68 | 69 | 70 | 71 | 21 |
| Migration time/min | 5.59 | 5.91 | 6.13 | 6.29 | 6.68 | 6.83 | 6.90 | 7.21 | 7.42 | 7.50 | 7.73 | 8.02 | 8.33 | 8.67 | 8.92 | 10.06 |
| R.S.D. (%) | 1.14 | 1.30 | 1.42 | 1.50 | 1.69 | 1.76 | 1.80 | 1.92 | 2.02 | 2.05 | 2.17 | 2.28 | 2.42 | 2.58 | 2.69 | 3.48 |

## 4. Conclusions

Acetic acid employed in this study not only is for control of running buffer pH but also participates complexation with REE ions. As an assistant complex-forming reagent, acetic acid plays an important role in improvement of separating Eu and Gd, and Y and Dy. With the combination of HIBA and HAc, an operational system has been developed for a complete separation of all 16 rare earth elements.

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