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# QUANTITATIVE ASPECTS OF RARE EARTH METAL DETERMINATIONS USING CAPILLARY ELECTROPHORESIS WITH INDIRECT ABSORBANCE DETECTION

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## ABSTRACT

The practical utility of capillary zone electrophoresis with indirect absorbance detection is examined for the separation and quantitation of rare earth metals. Various imidazole derivatives are investigated as to their suitability as running buffer (displaceable) detection ions with α-hydroxyisobutyric acid functioning as a chelating agent to enhance separations. Parameters important for quantitative analysis, such as limits of detection, relative standard deviation of peak areas, efficiency, resolution, peak shape and linear dynamic range are presented. The influences of sample matrix, method of injection, and background ion identity on these parameters are investigated and discussed.

## INTRODUCTION

Capillary zone electrophoresis (CZE) is a powerful tool in the separation and determination of a wide variety of analytes from small inorganic cations or anions to large peptides and proteins. CZE is usually performed in narrow bore open capillaries (typically 25 - 100 μm inner diameter and 50 to 100 cm long) filled with an aqueous running buffer solution and suspended between two buffer reservoirs. A high voltage (usually between 10 and 50 kV) is applied across the capillary causing the solutes to migrate through the capillary toward the detector (typically located at the cathodic end) via electroosmosis (1) and electrophoretic migration (2). High efficiency in CZE is attained due to efficient dissipation of electrophoretically generated heat by the diminutive capillary and the plug-like profile characteristic of electroosmotic flow. As a consequence, efficiency is generally limited only by longitudinal diffusion (2) and plate numbers often exceed 500,000 per meter (2,3).

Resolution in CZE is given by the equation

$$Rs = \frac{\sqrt{N}}{4}\left(\frac{\mu_1 - \mu_2}{\bar{\mu} - \mu_{osm}}\right) \qquad (1)$$

where N is the number of theoretical plates, $\mu_1$ and $\mu_2$ the electrophoretic mobilities of the solutes of interest, $\bar{\mu}$ the average of $\mu_1$ and $\mu_2$, and $\mu_{osm}$ the electroosmotic flow mobility. It has been shown that maximum resolution is attained when $\mu_{osm}$ is approximately equal and opposite in sign to $\bar{\mu}$, although at the expense of analysis time (2). This is often achieved by lowering the running buffer pH; Si-O$^-$ groups at the capillary walls are neutralized resulting in a lower zeta potential and, hence, a lower $\mu_{osm}$ (2). However, for solutes with very small differences in mobility, ($\mu_1$ - $\mu_2$), this manipulation of $\mu_{osm}$ may not provide adequate resolution even when high efficiency is attained.

An advantage of CZE is the ability to alter electrophoretic performance (e.g., improve selectivity) by adding various reagents to the running buffer. Specific interactions of solutes with reagents in the running buffer often enhance separations in CZE. Approaches to date include the addition of surfactants (4), organic solvents (5), and chelating agents (6), to name a few. Ideally, all chemical or physical interactions of injected solutes with system components occur exclusively with the species in solution, so novel reagents can be investigated without complications resulting from the preparation and use of stationary phases. In addition, the low volume of running buffer required in CZE facilitates the evaluation of rare and expensive substances for both applied and fundamental studies and minimizes disposal problems when toxic materials are involved.

A potential application for CZE is the separation and detection of rare earth metals. Two obvious concerns in this application are the similarity of lanthanide mobilities and their lack of suitable absorption bands for detection. The intrinsic mobilities range only from 72 to 67 x $10^{-5}$ $cm^2/V{\cdot}s$ across the period from $La^{3+}$ to $Lu^{3+}$ (7). This small difference does not allow sufficient resolution. In this work, an anionic ligand ($\alpha$-hydroxyisobutyric acid, HIBA) was added to the running buffer. The HIBA forms weak reversible complexes with the lanthanides. This serves to differentially reduce the positive mobilities of the metal ions and, thereby, magnifies differences in mobility.

The lack of suitable absorption bands for the lanthanides requires an alternative to direct absorbance detection which is the most common mode of detection in CZE. Indirect absorption is a universal detection method that permits the analysis of analytes which do not absorb at the selected wavelength (8). An ionic, highly absorbing ion is added to the running buffer and is displaced by the analyte due to charge repulsion. The signal, therefore, is caused by the displaceable ion and the analyte is seen as a negative peak due to the absence of that ion in the analyte band.

The mobility of the background ion is critical to the efficient separation of the analytes (7, 9-13). If the mobility of the background ion is greater than that of the analyte, the peak will "tail." Conversely, if the mobility of the background ion is lower than that of the analyte, peak "fronting" will be observed. This peak asymmetry will adversely effect the efficiency and resolution of the separation and the limit of detection (LOD) and linear dynamic range (LDR) of the analyte measurement. The ionic strength of the sample matrix is also important and can effect peak shape, again degrading the analytical parameters. Injection technique, whether based on electrokinetic or hydrostatic methods (14, 15), will effect the asymmetry, efficiency, LOD, reproducibility, and resolution. The goal of these experiments is to study the effects of these factors on quantitative figures of analytical merit.

## EXPERIMENTAL

### Apparatus

The CZE separation apparatus was similar to that previously described (6) and consisted of the following: electrical fields are provided with a Spellman high voltage power supply (Plainview, NY) electrically connected to inlet and outlet reservoirs (microcentrifuge vials) by using platinum wire electrodes. Fused silica capillaries (75 μm i.d. x 365 μm o.d.) surface modified with a C-8 phase were supplied by Suppelco (Bellefonte, PA).

Ultraviolet absorbance detection at 214 nm was accomplished using a SSI (Scientific Systems Inc, State

College, PA) Model 504 absorbance detector with a CZE flow-cell adapter. The electropherograms were recorded on a Varian (Palo Alto, CA) Model 4400 integrator.

## Chemicals

The samples were various rare earth nitrates or oxides purchased from *Sigma (St. Louis, MO)* or *Aldrich (Milwaukee, WI)*. The oxides were dissolved in concentrated nitric acid and evaporated to obtain the nitrates. The running buffer was HPLC grade water from Baxter Scientific containing 10 mM of various imidazole derivatives obtained from Aldrich and 3 mM HIBA purchased from Sigma adjusted to a pH of 4.7 using acetic acid. The samples were dissolved in either water or running buffer.

## Procedures

The capillaries used herein were cut to 50 cm lengths and an optical window produced by removing the polyimide coating 10 cm from one end of the capillary with a hot 50% sulfuric acid solution and rinsing with water. A syringe needle was affixed to the opposite (inlet) end to facilitate rinsing.

The capillaries were initially treated with 0.01 M NaOH for a period of 10 minutes to remove impurities from the surfaces and rinsed with HPLC grade water. The capillaries were then filled with the running buffer and suspended between the two running buffer reservoirs which were positioned at equal height to eliminate hydrostatic flow. The operating voltage (15 kV) was applied across the capillary and the system allowed to equilibrate for thirty minutes.

Sample injections were made either hydrostatically or electrokinetically. Hydrostatic injections were accomplished by placing the inlet end of the capillary in the sample solution and raising it 10 cm above the outlet end for 26 sec. The capillary was then removed from the sample, rinsed with HPLC grade water, placed in the inlet buffer reservoir, and the separation voltage was applied. Electrokinetic injections involved replacing the inlet reservoir with the sample container and applying a potential of 2 kV across the capillary for a period of 26 sec. The capillary inlet was then rinsed and returned to the buffer reservoir prior to applying the separation voltage (14, 15).

## Calculations

The electropherograms were recorded at a high chart speed on the integrator and measured by hand for area, efficiency, resolution, and asymmetry. This was done manually due to the tailing nature of the peaks which "confused" the integrator. The equations used for each calculation are given below.

$$\mathbf{A = \frac{1}{2}(bh)} \qquad (2)$$

where the peaks were triangulated and the area (A) was calculated using the base (b) and height (h).

$$\mathbf{N = 5.54\left(\frac{t_r}{w_{1/2}}\right)^2} \qquad (3)$$

where the efficiency (N) is related to the retention time ($t_r$) of the analyte of interest and the width of the peak at half height ($w_{1/2}$).

$$\mathbf{Rs = \frac{t_{r2} - t_{r1}}{w_{h2}}} \qquad (4)$$

where the resolution (Rs) is related to the difference in retention times of the two analytes of interest and the baseline width ($w_b$) of the second.

$$As = \frac{w_r}{w_l} \qquad (5)$$

where the asymmetry (As) is calculated by dropping a vertical from the peak center and dividing the width of the right ($w_r$) part by the width of the left ($w_l$) part at 10% of the peak height.

## RESULTS AND DISCUSSIONS

### Background Ion Mobility

Imidazole was chosen to be the background ion because it was reported to have a mobility that matched those of the rare earth metals (7, 13). Moreover, imidazole absorbs strongly at 214 nm at pH 4.7 and does not interact with either the capillary walls or the analytes. However, it was observed in this work that its mobility was greater than the rare earth analytes and, consequently, its use resulted in badly tailing peaks. Other imidazole derivatives were investigated with the goal of finding one with a lower mobility to improve efficiency and asymmetry (Refer to Table 1). Of the five imidazoles investigated, only 2-ethylimidazole and 2-benzyl-2-imidazoline seemed reasonable. However, the baseline for 2-benzyl-2-imidazoline was very noisy and gave extraneous peaks in the vicinity of the peak for lanthanum. The baseline for 2-ethylimidazole was much quieter and the efficiency decreased gradually for the later analytes whereas the efficiency decreased drastically for other imidazole derivatives (i.e., the mobility match was better for 2-ethyl

**Table 1**: COMPARISON OF EFFICIENCY[a]/ASYMMETRY[b] FOR TESTED BACKGROUND IONS[c]

| Background ion | La | Pr | Eu | Tb | Dy |
|---|---|---|---|---|---|
| Imidazole | 17/5.0 | 14/5.8 | 13/6.3 | 11/5.7 | 11/4.9 |
| 2-Methylimidazole | 34/4.0 | 23/4.7 | 18/5.8 | 17/5.4 | 14/5.0 |
| 4-Methylimidazole | 27/4.6 | 17/5.9 | 14/6.3 | 12/6.1 | 14/6.8 |
| 2-Ethylimidazole | 37/3.4 | 28/4.8 | 24/5.4 | 21/5.0 | 24/4.9 |
| 2-Benzyl-2-imidazoline | 46/1.9 | 99/4.6 | 31/6.3 | 19/5.9 | 27/4.4 |

a) N by equation 3 divided by 1000
b) As by equation 5
c) Injected concentration of rare earth metals 50 ppm

imidazole). This, then, was chosen as the background ion for these experiments.

## Chelating Agent Concentration

Experiments were conducted to determine the appropriate HIBA concentration. This acid differentially complexed the lanthanides, improving selectivity and resolution. Based on experiments using 1 to 9 mM HIBA concentrations in the running buffer, it was determined that 3 mM HIBA gave sufficient resolution. A higher concentration was not used to prevent excessive Joule heating due to a higher running buffer ionic strength. The effect of HIBA concentrations on these rare earth separations is demonstrated in Figure 1.

## Sample Matrix

The sample consisted of La, Pr, Eu, Tb, and Dy nitrates dissolved in water and diluted to the desired concentration in

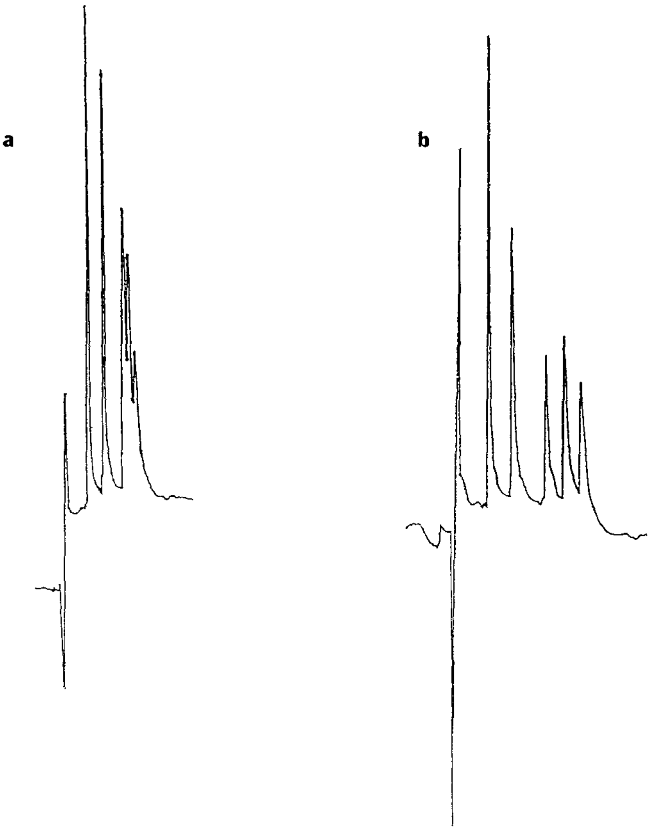

FIGURE 1. Electropherograms using (a) 1 mM HIBA (b) 3 mM HIBA. Running buffer contained 10 mM 2-ethylimidazole and the sample contained 50 ppm each of La, Pr, Eu, Tb, and Dy nitrates (in order of elution following -/+ solvent disturbance) dissolved in water.

either water or running buffer. The plug length injected (see Experimental Section) onto the column was about 5 mm. Such a large volume (28 nL) was necessary to detect moderately low concentrations. Large injection volumes or analyte concentrations can cause a decrease in efficiency (2). However, the poor match in mobilities between the background ion and the analyte ion was the predominate cause of tailing and poor efficiency in this work.

With a sample matrix of water, injection stacking is generally observed (14 - 16). Stacking occurs when a sample of low ionic strength is injected into a running buffer of higher ionic strength. When the voltage is applied, the field within the analyte band is higher due to fewer ions and a higher resistivity. The ions move toward the front of the band where they encounter the running buffer and a lower field. The result is a stacking (or concentrating) of the sample ions at the front of the band. When the sample matrix is the running buffer or a solution of comparable ionic strength, the stacking mechanism is not operative. The stacking process can dramatically improve detectability and is necessary to see moderately low concentrations (see Table 2 and the related discussion below).

Lanthanide LODs in the sub-ppm range have been reported with the assistance of sample stacking (7, 12). Unfortunately, the practical utility of this process is questionable since most rare earth metal analyses involve matrices that have moderate to high ionic strength. Thus, there remains a need either to [1] develop methodologies to selectively salt-out (perhaps via extraction or precipitation) extraneous ionic components in realistic rare earth samples prior to injection or to [2] improve current detection methods for CZE.

## Injection Method and Matrix

The method of injection, in combination with the sample matrix, has a profound effect on the LOD and reproducibility of the separation. In this work, electrokinetic injections from a sample matrix of water provided better detectability than other methods and matrices. The lowest detectable concentration injected was approximately 0.5 ppm.

Unfortunately, both the peak area and height reproducibility observed with this method were extremely poor. The analytes stack during the injection (sometimes dubbed field amplified injection (15)) as well as during the early stages of the separation. While this provides for a considerable concentrating effect, it also means that slight variations in time, applied voltage, position of the capillary within the sample, etc., have large effects on the injection process (15). When the calibration curve for Pr is plotted for this method, a regression coefficient of 0.080 is obtained (see Figure 2a). This can be compared with a regression coefficient of 0.999 for Pr for hydrostatic injections from water (see Figure 2b). Thus, electrokinetic injection from water is very sensitive but appears to lack the precision required for quantitative work.

Table 2 summarizes results obtained in this work concerning the effects of injection method and sample matrix on efficiency, asymmetry, limits of detection, and peak area reproducibility. Many different influences act on these parameters in a complex interplay such that trends due to one influence or another are difficult to discern. One observation regarding Table 2 is that differences in mobilities between the background ion and analytes leads to a large asymmetry factor which degrades other parameters. Measuring the tailing peaks is challenging because the beginning and end of the peaks are difficult to determine; therefore, higher RSDs are obtained. Moreover, this uncertainty in measuring peaks creates a comparable uncertainty in the determination of other parameters in Table 2. The asymmetry also decreases efficiency and increases LODs.

Certain points regarding the efficiency data in the table can be made. Long injection plugs can limit the efficiency; the peak variance due to the injection ($\sigma^2_{inj}$) should be less than ten percent of the $\sigma^2_{tot}$. The long plugs used in these experiments probably limited efficiency when injections were made from a running buffer matrix (see entries "f" and "g" in

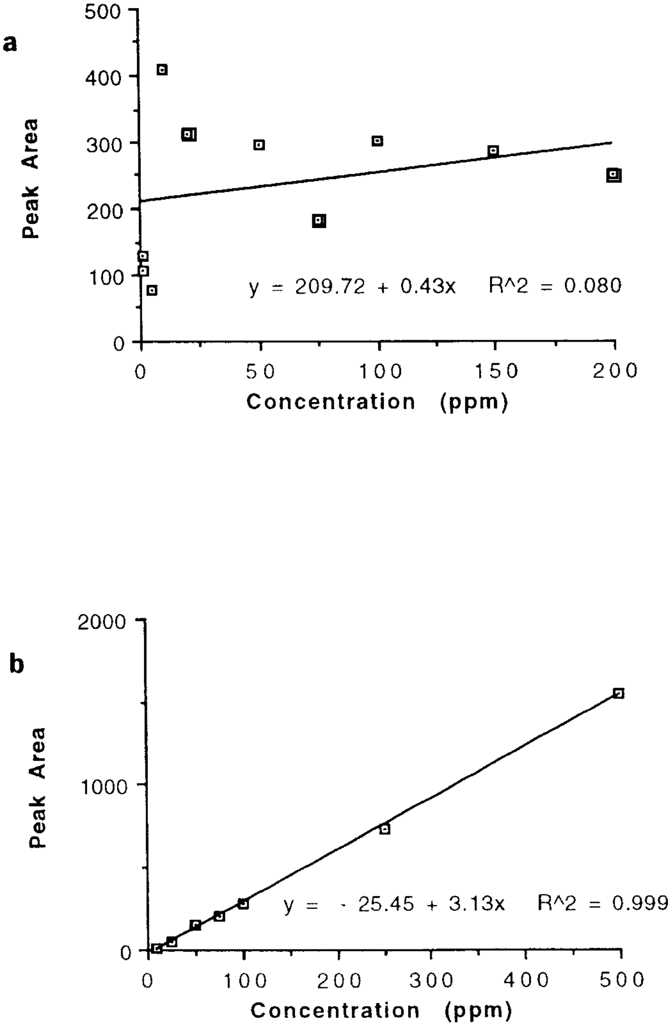

FIGURE 2. Calibration curves for (a) electrokinetic injection from water (lowest injected/detected concentration 0.5 ppm) and (b) hydrostatic injection from water matrix (lowest injected/detected concentration 10 ppm). Conditions as in Figure 2b.

**Table 2**: FIGURES OF MERIT
EFFICIENCY, ASYMMETRY, LIMIT OF DETECTION WITH RSDs

| Rare Earth | Efficiency[a] | As[b] | LOD (ppm)[c] | RSD of areas[d] |
|---|---|---|---|---|
| | e/f/g | e/f/g | e/f/g | e/f/g |
| La | 79/13/78 | 4.3/2.7/9.0 | 1.7/2.9/7.9 | 17/5.8/13 |
| Pr | 28/11/15 | 8.7/7.3/8.3 | 2.7/2.9/13 | 13/7.0/19 |
| Eu | 11/10/13 | 11/10/7.1 | 3.9/7.7/27 | 12/5.7/32 |
| Tb | 19/6.9/14 | 13/8.5/7.1 | 4.1/15/43 | 14/6.0/26 |
| Dy | 20/4.0/19 | 10/6.0/6.0 | 5.4/24/50 | 14/13/42 |

a) N by equation 3 divided by 1000 at 50 ppm
b) As by equation 5 at 50 ppm
c) LOD at S/N = 3 using peak height
d) RSD determined at 50 ppm with n = 6 - 10
e) hydrostatic injection from a water matrix
f) hydrostatic injection from a running buffer matrix
g) electrokinetic injection from a running buffer matrix

Table 2). The stacking (see discussion above) that occurs for injection from water offsets this problem (i. e., efficiency is generally better for the "e" entries in Table 2). The stacking effect has its limits, however, as a hydrostatic (or parabolic) flow caused by the mismatch between the sample band and the bulk electroosmotic velocity can ultimately lead to band dispersion. Thus, an optimum plug length exists (14, 15).

The hydrostatic injections from water are fairly well behaved and produce reasonable efficiency and the best LODs. The peak asymmetry for this mode of injection is somewhat puzzling. The stacking process increases the ionic strength of the sample zone within the capillary. While highly concentrated samples can produce field distortions and peak asymmetry (2), the stacking process should terminate before this occurs. However, the concentration of the stacked sample for this matrix and method of injection is clearly greater than

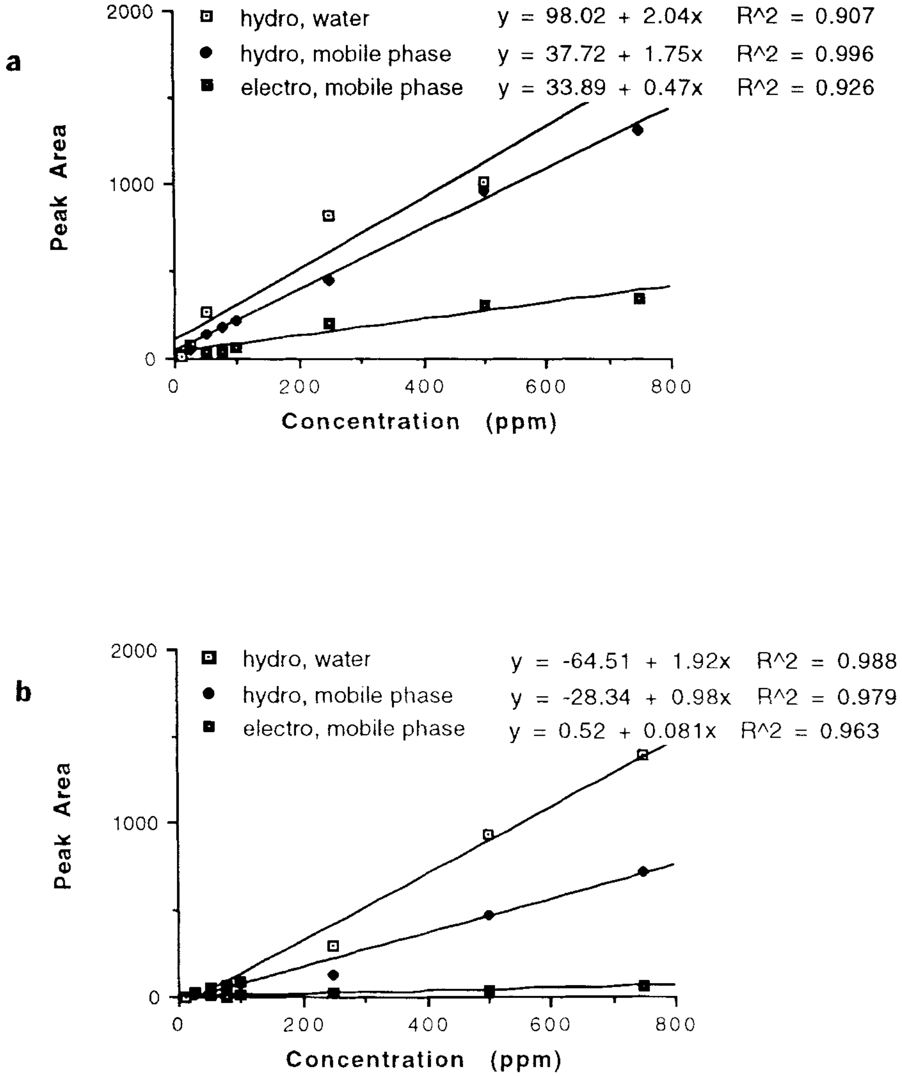

FIGURE 3. Calibration curves for a) La and b) Dy using hydrostatic injections from water, hydrostatic injections from running buffer, and electrokinetic injections from running buffer. Conditions as in Figure 1b.

50 ppm (i. e., greater than for the other modes of injection appearing in the table), and it is possible that this exacerbates the asymmetry problem caused by differences in mobility between background ion and rare earth ion.

Figure 3 shows the calibration curves for La and Dy, the first and last metals to elute. The linear dynamic range for each method studied was up to two decades of concentration above the LOD except in the case of La in the hydrostatic injections from water. That plot showed a roll-off due to overloading the concentration of 2-ethylimidazole in the running buffer. In every other case, the upper boundary of the LDR was determined by a lack of resolution at high injected concentration and not by limitations in the linearity of the response.

## CONCLUSIONS

Sample stacking is necessary to achieve moderate LODs. Realistic samples, unless relatively concentrated, are not likely to be determined quantitatively using this technique due to the low ionic strength requirement of stacking. This methodology is not optimized at this time for quantitation of rare earth metals due to modest efficiency and, more significantly, poor detectability. Based on these results, future studies will include the use of higher HIBA concentrations and a background ion with lower mobility. Ultimately, alternate modes of detection and/or on-column sample concentration will be investigated and separation of actinides will be attempted.

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