

## Deferitazole, a New Orally Active Iron Chelator

### Supporting Information

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1. Determination of Log  $D_{7.4}$  Values for Deferitazole Ligand and [(Deferitazole)<sub>2</sub>Fe]<sup>-</sup>
2. Fe/edta Competition Study
3. Determination of the Redox Potential for the Iron Complex of Deferitazole using Cyclic Voltammetry
4. Determination of Log  $K$  Values for Divalent Metals: Ca<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>, Pb<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup>
5. MS Study of Deferitazole Divalent Metal Complexes
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## 7. Determination of Log $K$ Values for Trivalent Metals: $\text{Al}^{3+}$ and $\text{La}^{3+}$

### 1. Determination of Log $D_{7.4}$ Values for Deferitazole Ligand and $[(\text{Deferitazole})_2\text{Fe}]^-$

**Table S1.** Log  $D_{7.4}$  values for replicate measurements of deferitazole species

	$A_0$	$A_1$	$V_{\text{aqu}}$	$V_{\text{oct}}$	$D_{7.4}$	$\log D$	Mean	SD
Deferitazole	0.354 22	0.287 61	1.000	6.000	0.039	-1.413	-1.29	± 0.13
Deferitazole	0.354 22	0.293 56	1.000	4.000	0.052	-1.287		
Deferitazole	0.354 22	0.310 81	1.000	2.000	0.070	-1.156		
Deferitazole iron(III) complex	0.954 76	0.943 77	1.000	4.000	0.003	-2.536	-2.68	± 0.17
Deferitazole iron(III) complex	0.954 76	0.946 08	1.000	4.000	0.002	-2.639		
Deferitazole iron(III) complex	0.954 76	0.953 47	1.000	1.000	0.001	-2.869		
Deferitazole iron(III) complex	0.953 47	0.901 08	1.000	6.000	0.010	-2.014		

SD, standard deviation.

Octanol-saturated MOPS buffer  $V_{\text{aq}}$  mL containing deferitazole or deferitazole iron(III) complex was mixed with  $V_{\text{oct}}$  mL water-saturated octanol.

Solutions were shaken vigorously for 10 min at room temperature; the absorbance of the aqueous phase was then monitored at peak  $\lambda_{\text{max}}$ .

The following formula was adopted for log  $D$  calculation:

$$P = (A_0 - A_1)V_w/A_1V_o$$

where  $A_0$  = initial absorbance of the octanol phase;  $A_1$  = absorbance at equilibrium of octanol phase after the addition of aqueous phase;  $V_w$  = volume of the octanol; and  $V_o$  = total volume of aqueous phase. For deferitazole  $n = 3$  and for the deferitazole iron(III) complex  $n = 4$ .

## 2. Fe/edta Competition Study

The exchange rate of iron between deferitazole and edta is slow (Figure S1). Consequently competition incubations were run overnight (Figure S2). Analysis of this study gave a  $\log \beta_2 = 33.39 \pm 0.031$  which is in close agreement with the titration data.

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EDTA competition

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$$\beta_2 = 33.39 \pm 0.031$$

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Fe<sup>III</sup> edta constants taken from:

Anderegg G; (1977);

*Critical Survey of*

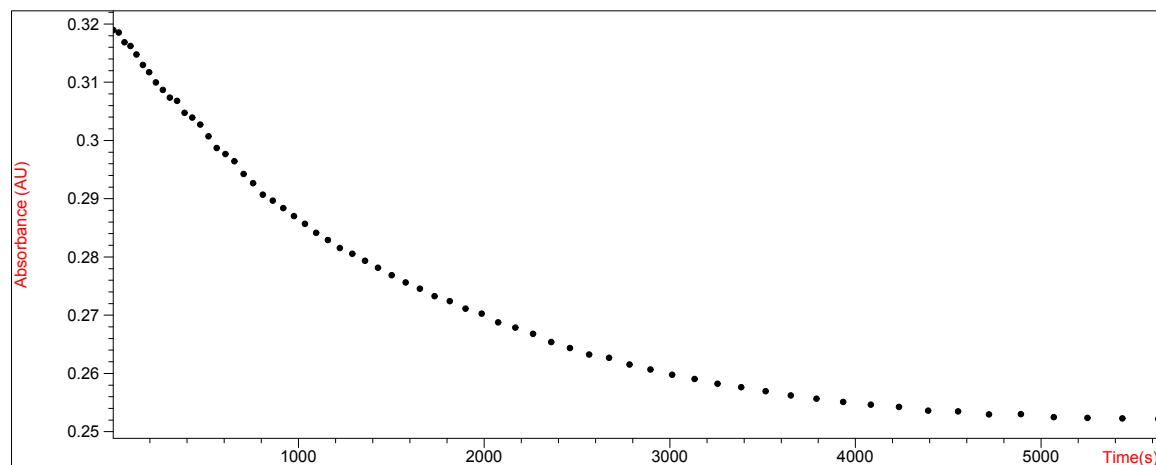
*Stability Constants of*

*EDTA Complexes;*

IUPAC Chemical Data

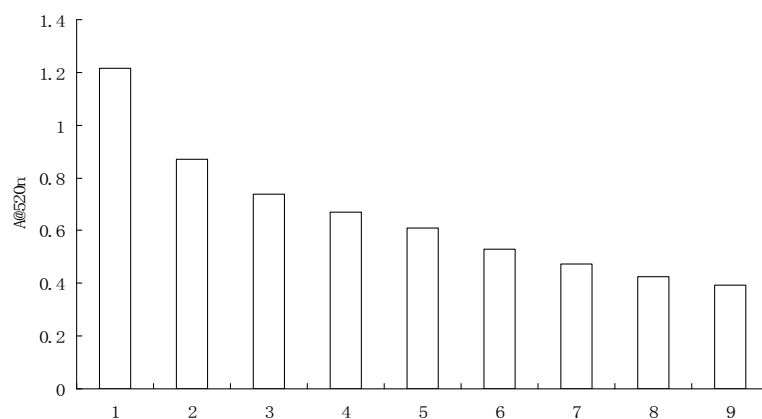
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Press, Oxford



**Figure S1.** Kinetic time course of deferitazole iron(III) complexes edta competition at 520 nm.

[iron] = 87  $\mu$ M; [deferitazole] = 350  $\mu$ M; [edta] = 2mM at pH 7.45 (0.1 M MOPS).

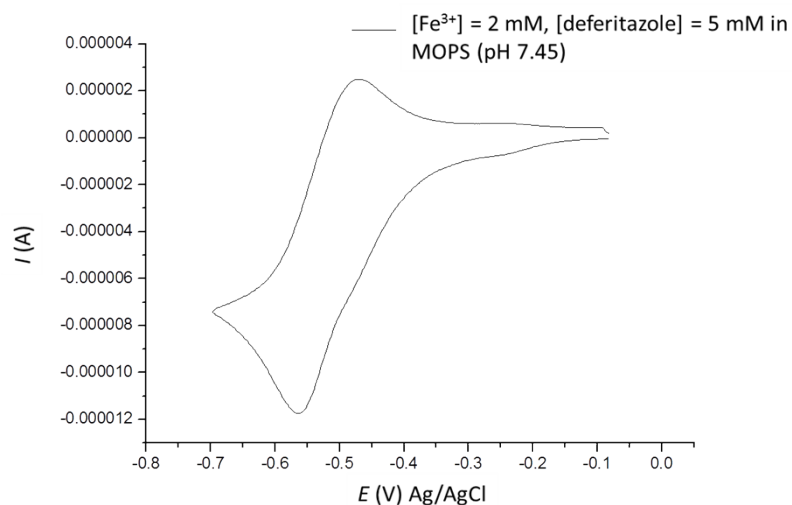


**Figure S2.** Absorbance of competition results at 520 nm with various edta concentrations at pH 7.45 (0.1 M MOPS).

[iron] = 68.8  $\mu$ M; [deferitazole] = 207  $\mu$ M; [edta] = 0, 4.24, 8.47, 12.71, 16.95, 21.19, 25.42, 29.66, 33.90 mM.

### 3. Determination of the Redox Potential for the Iron Complex of Deferitazole using Cyclic Voltammetry

Cyclic voltammetry measurements were performed with a CS-120 device (Corrtest). A concentrated solution of deferitazole (5 mM) and iron(III) chloride (2 mM) at pH 7.42 (0.2 M MOPS buffer) were prepared. All measurements were conducted under nitrogen in a jacketed, one-compartment cell with a platinum disk working electrode (geometric area: 0.07 cm<sup>2</sup>) (Corrtest), a platinum wire counter electrode (Corrtest) and an Ag/AgCl reference electrode. The working electrode surface was polished, sonicated in water, and air-dried immediately before use. The sweep rate was 100 mV/s. Oxygen was removed from the electrolyte solution by bubbling nitrogen through the solvent for several minutes prior to making the measurement.



**Figure S3.** Cyclic voltammetry of deferitazole.iron complexes.

Cathodic and anodic peaks were located at  $-0.567 \text{ V}$  and  $-0.472 \text{ V}$  vs Ag/AgCl ( $n = 2$ ). The redox potential of deferitazole iron complexes is calculated as  $E_{1/2} = (E_{\text{cathodic}} - E_{\text{anodic}}) = -519.5 \text{ mV}$ , therefore a normal hydrogen electrode potential will be  $-322 \text{ mV}$  at pH 7.45.

Using these data together with the stability constant acquired from spectrophotometric titration, the affinity constant for iron(II) was calculated from Equation S1, which is

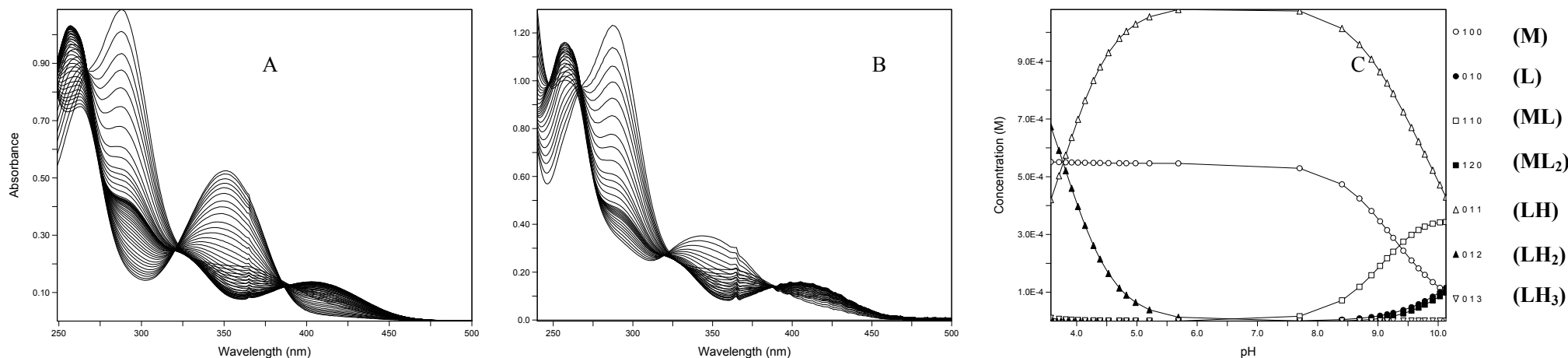
derived from the Nernst equation:

$$59.16(\log \beta[\text{Fe}^{3+}] - \log \beta[\text{Fe}^{2+}]) = E(\text{Fe}^{3+}/\text{Fe}^{2+}) - E_{\text{complex}}(\text{Fe}^{3+}/\text{Fe}^{2+}) \quad \text{Equation S1}$$

where  $E(\text{Fe}^{3+}/\text{Fe}^{2+})$  is the redox potential of the iron pair in the absence of the ligand and  $E_{\text{complex}}(\text{Fe}^{3+}/\text{Fe}^{2+})$  is the redox potential in the presence of the ligand. The calculated value  $\log \beta_2 = 14.8$  for iron(II).

#### 4. Determination of log *K* Values for Divalent Metals: Ca<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>, Pb<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup>.

##### Log *K* for Ca<sup>2+</sup>



**Figure S4 (A).** Experiment with [deferitazole] = 1102.8 μM; [Ca<sup>2+</sup>] = 551.3 μM; ratio of L:M = 2; start in 20.315 mL 0.1 M potassium chloride at 25 °C.

pH from 3.572 to 10.836; total points 34. (B) Experiment with [deferitazole] = 986 μM; [Ca<sup>2+</sup>] = 986.3 μM; ratio of L:M = 1; start in 20.277 mL 0.1 M potassium chloride at 25 °C. pH from 3.598 to 10.918; total points 45. (C) Speciation plot corresponding to conditions in (A).

Comment: complexes start to form only at pH higher than 9.

**Table S2.** Calculated affinity constants for Zn(II).deferitazole complexes

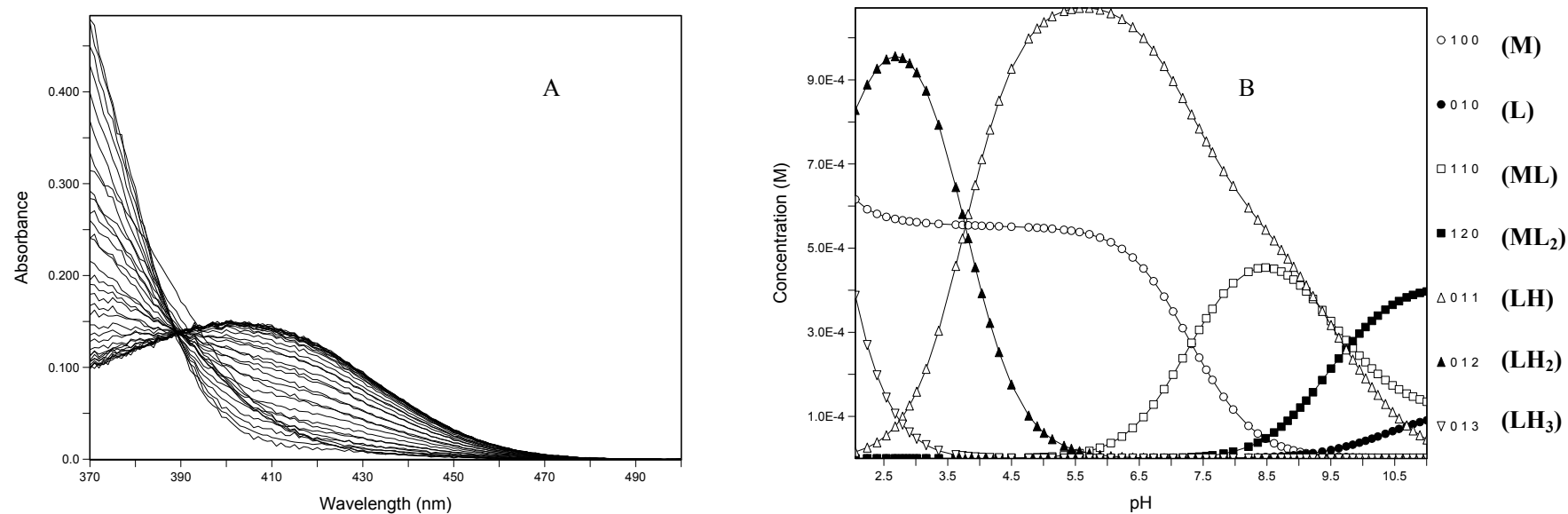
For 1:1 experiment	For 1:2 experiment
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$$\text{Log } K_1 = 4.47 \pm 0.018 \quad \text{Log } \beta_2 = 7.86 \pm 0.044$$

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## Log $K$ for $Mg^{2+}$



**Figure S5** (A). Experiment with [deferitazole] = 1230.9  $\mu$ M; [ $Mg^{2+}$ ] = 615.5  $\mu$ M; ratio of L:M = 2; start in 20.414 mL 0.1 M potassium chloride at 25 °C. pH from 2.053 to 11.003; total points 67. (B) Speciation plot corresponding to conditions in (A).

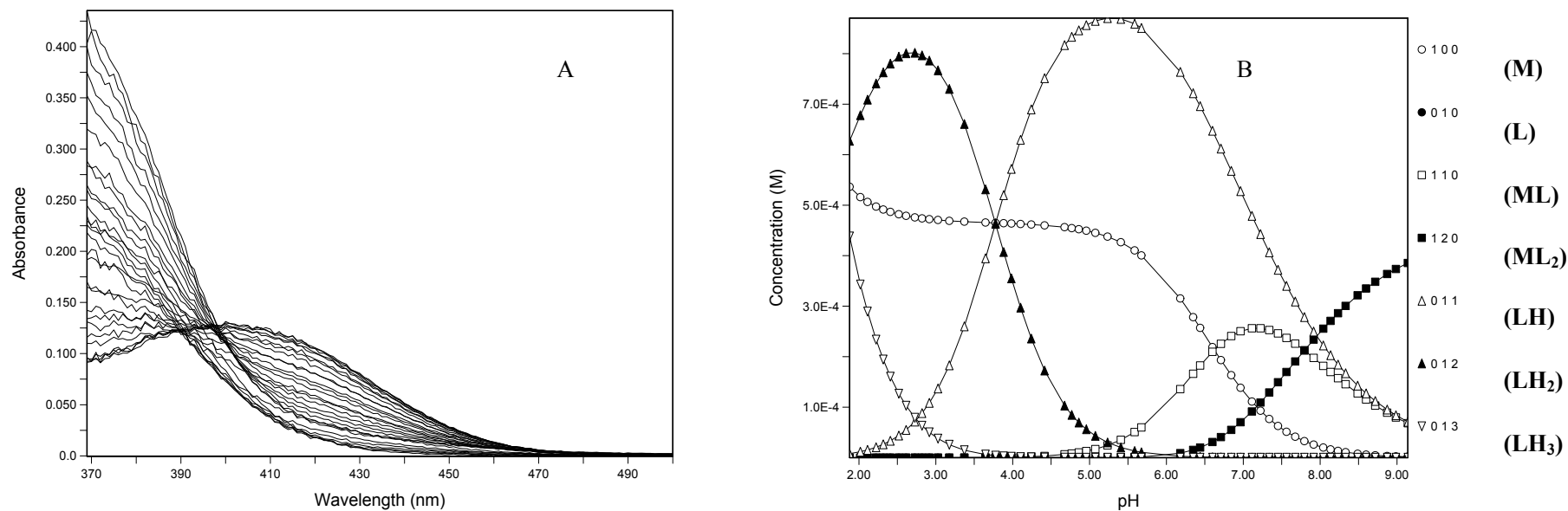
**Table S3.** Structures of Mg(II).deferitazole complexes and calculated affinity constants

ML <sub>2</sub>	ML	Affinity (log)
		$K_1 = 5.951 \pm 0.056$
		$\beta_2 = 10.198 \pm 0.066$





## Log $K$ for $Mn^{2+}$

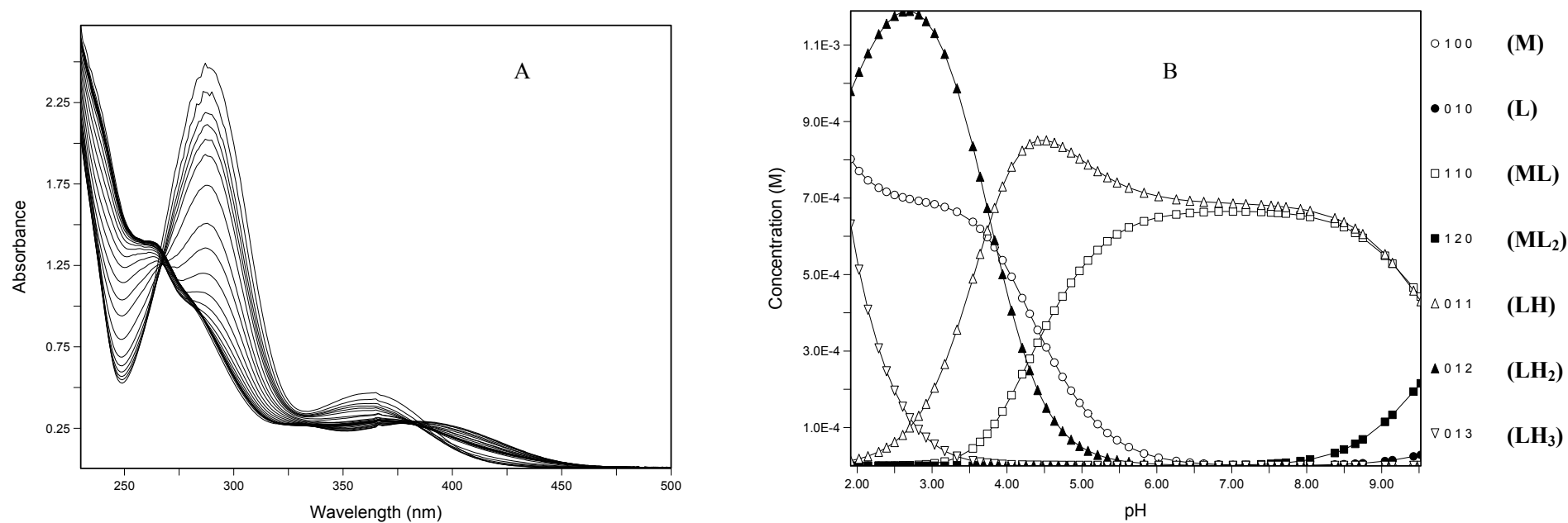


**Figure S6** (A). Experiment with [deferitazole] = 1073.6  $\mu$ M; [ $Mn^{2+}$ ] = 536.3  $\mu$ M; ratio of L:M = 2, start in 20.771 mL 0.1 M potassium chloride at 25  $^{\circ}$ C. pH from 1.878 to 10.802; total points 69. (B) Speciation plot corresponding to conditions in (A).

**Table S4.** Structures of Mn(II).deferitazole complexes and calculated affinity constants

$ML_2$	$ML$	Affinity (log)
		$K_1 = 7.269 \pm 0.057$
		$\beta_2 = 13.73 \pm 0.089$

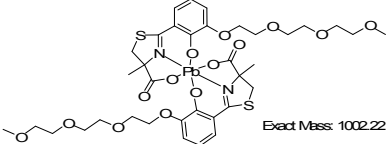
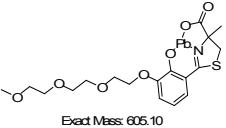
## Log $K$ for $\text{Pb}^{2+}$



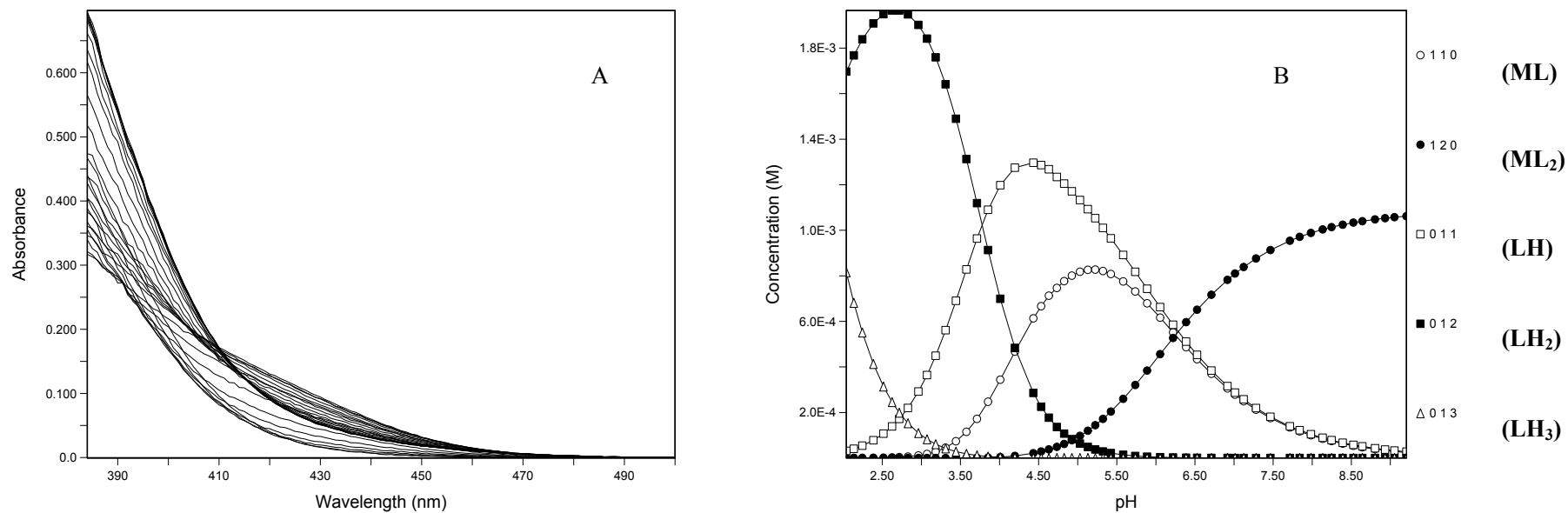
**Figure S7 (A).** Experiment with [deferitazole] = 1624.3  $\mu\text{M}$ ; [ $\text{Pb}^{2+}$ ] = 802.1  $\mu\text{M}$ ; ratio of L:M = 2; start in 20.174 mL 0.1 M potassium chloride at 25 °C. pH from 1.913 to 9.855; total points 56. (B) Speciation plot corresponding to conditions in (A).

Comment:  $\beta_2:K_1$  ratio is much lower than that observed with other metals.

**Table S5.** Structures of Pb(II).deferitazole complexes and calculated affinity constants

ML <sub>2</sub>	ML	Affinity (log)
 Exact Mass: 1002.22	 Exact Mass: 605.10	$K_1 = 9.318 \pm 0.010$ $\beta_2 = 13.551 \pm 0.077$

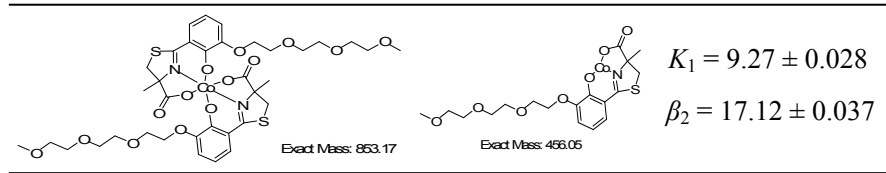
### Log $K$ for $\text{Co}^{2+}$



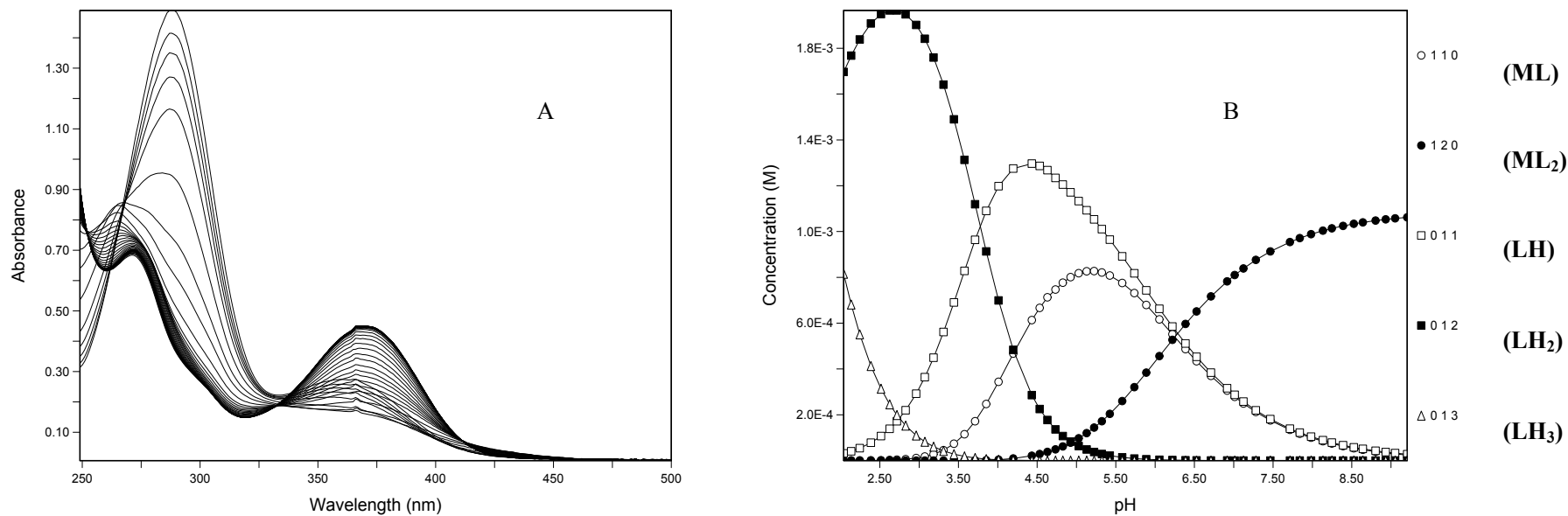
**Figure S8** (A). Experiment with [deferitazole] = 2543.9  $\mu\text{M}$ ;  $[\text{Co}^{2+}] = 1269.5 \mu\text{M}$ ; ratio of L:M = 2; start in 21.814 mL 0.1 M potassium chloride at 25 °C. pH from 2.040 to 9.053; total points 53. (B). Speciation plot corresponding to conditions in (A).

**Table S6.** Structures of Co(II).deferitazole complexes and calculated affinity constants

$\text{ML}_2$	ML	Affinity (log)
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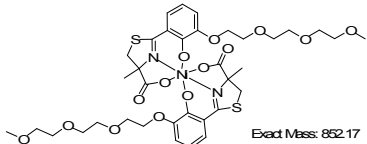
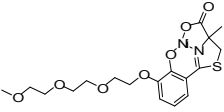


### Log $K$ for $\text{Ni}^{2+}$

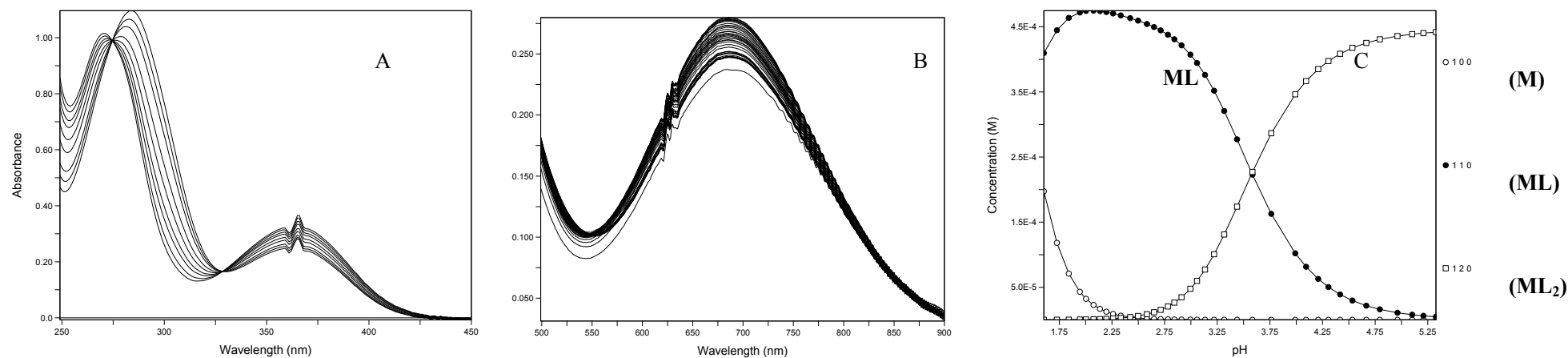


**Figure S9** (A). Experiment with [deferitazole] = 1018.2  $\mu\text{M}$ ;  $[\text{Ni}^{2+}] = 495.1 \mu\text{M}$ ; ratio of L:M = 2.1; start in 20.690 mL 0.1 M potassium chloride at 25 °C. pH from 2.055 to 10.895; total points 72. (B) Speciation plot corresponding to conditions in (A).

**Table S7.** Structures of Ni(II).deferitazole complexes and calculated affinity constants

ML <sub>2</sub>	ML	Affinity (log)
		$K_1 = 10.157 \pm 0.012$
Exact Mass: 862.17	Exact Mass: 466.06	$\beta_2 = 18.354 \pm 0.020$

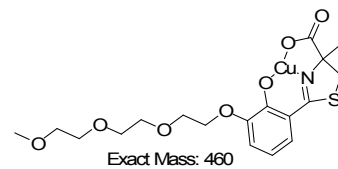
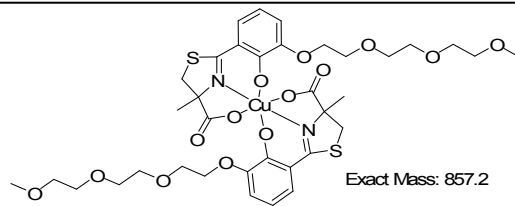
### Log *K* for Cu<sup>2+</sup>



**Figure S10** (A). Experiment with [deferitazole] = 82.2  $\mu$ M; [Cu<sup>2+</sup>] = 79.9  $\mu$ M; ratio of L:M = 1; start in 20.186 mL 0.1 M potassium chloride at 25 °C. pH from 1.752 to 3.075; total points 10. (B) Experiment with [deferitazole] = 1831.2  $\mu$ M; [Cu<sup>2+</sup>] = 607 M; ratio of L:M = 3; start in 15.943 mL 0.1 M potassium chloride at 25 °C; pH from 1.603 to 10.998; total points 77; (C).Speciation plot corresponding to conditions in (B).

**Table S8.** Structures of Cu(II).deferitazole complexes and calculated affinity constants

ML <sub>2</sub>	ML	Affinity (log)
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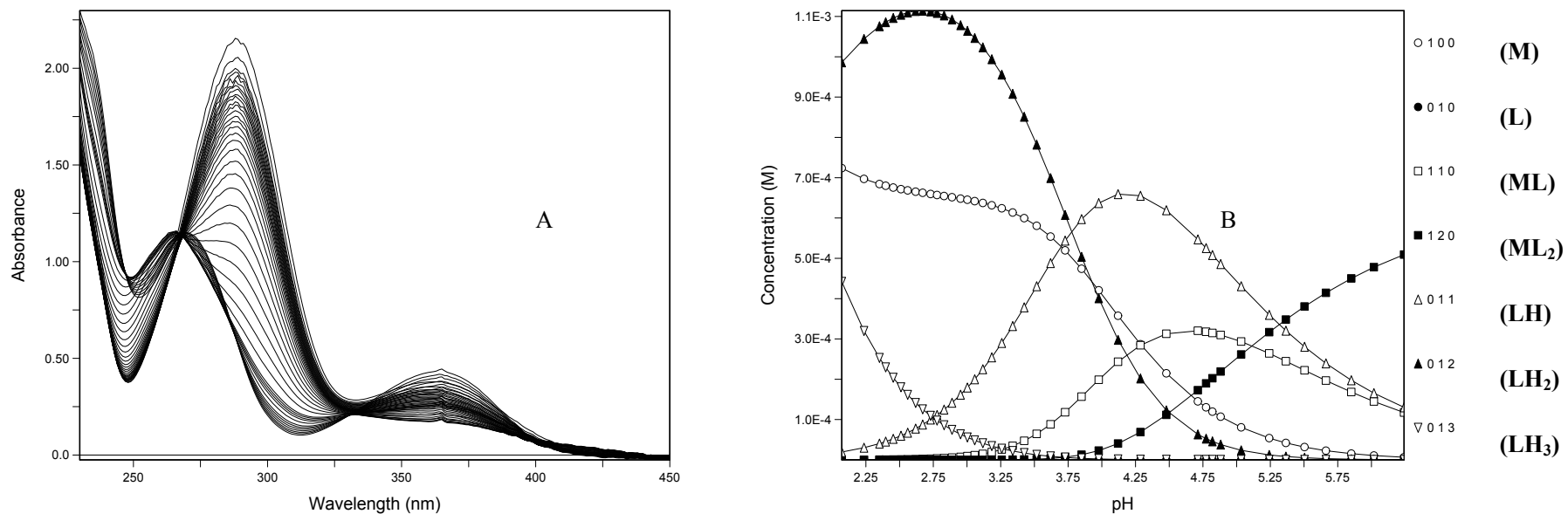


$$K_1 = 14.799 \pm 0.037$$

$$\beta_2 = 25.5 \pm 0.062$$



### Log K for Zn<sup>2+</sup>



**Figure S11** (A). Experiment with [deferitazole] = 1447.4  $\mu\text{M}$ ;  $[\text{Zn}^{2+}] = 723.6 \mu\text{M}$ ; ratio of L:M = 2; start in 21.133 mL 0.1 M potassium chloride at 25 °C. pH from 2.071 to 10.213; total points 50. (B) Speciation plot corresponding to conditions in (A)

**Table S9.** Structures of Zn(II).deferitazole complexes and calculated affinity constants

ML <sub>2</sub>	ML	Affinity (log)
		$K_1 = 9.593 \pm 0.045$
		$\beta_2 = 18.574 \pm 0.079$

## 5. MS Study of Deferitazole Divalent Metal Complexes

We investigated both positive and negative mode ESI MS for  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ . and in all cases we were able to identify the presence of both the ML and  $\text{ML}_2$  species. Because  $\text{Ca}^{2+}$  has an extremely low affinity for deferitazole, much higher concentrations of the ligand would be required than for the other studies. This was not undertaken to avoid potential damage to the instrument.

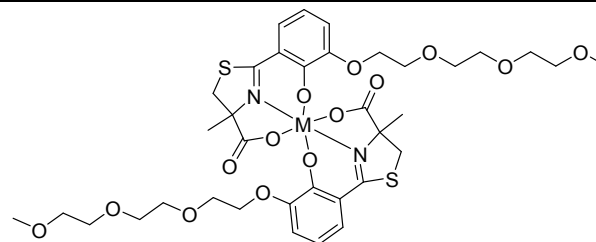
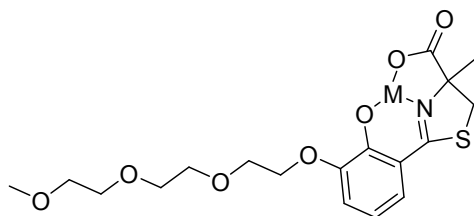
With  $\text{Mn}^{2+}$  we detected only peaks corresponding to  $\text{Mn}^{3+}$ ; this was caused by the oxidation of  $\text{Mn}^{2+}$  during the MS ionization process.

Generally in positive mode we identified proton adducts of ML that have one positive charge.

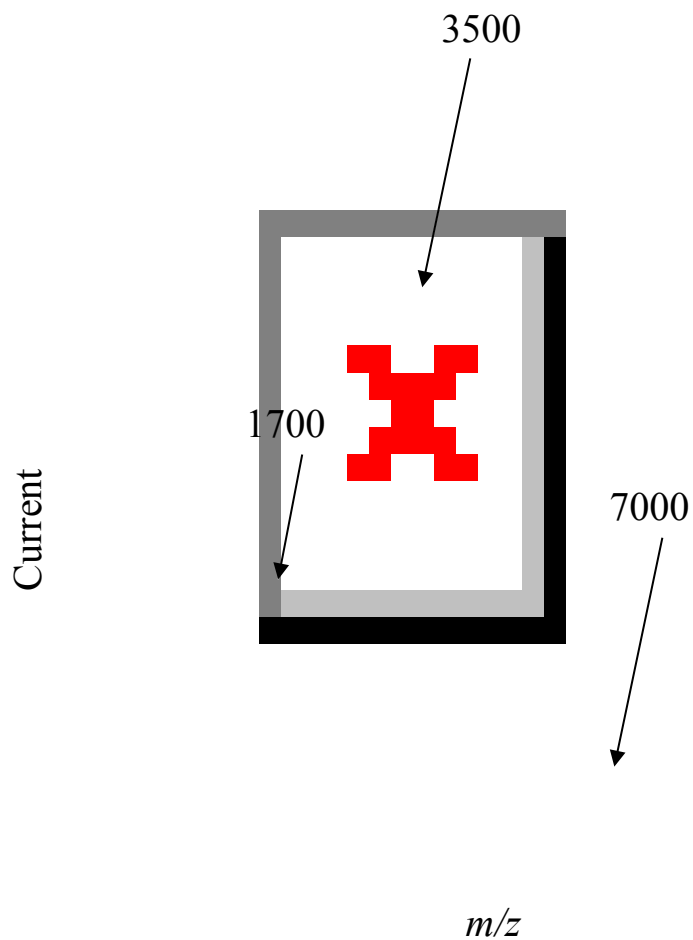
Samples prepared for MS were: MeOH 400  $\mu\text{L}$  + deferitazole (380  $\mu\text{M}$  in DMSO) 38  $\mu\text{L}$  + corresponding metal chloride salt solution in the molar ratio M:L = 1:2; 200  $\mu\text{L}$  were taken and added to 30  $\mu\text{L}$  0.1 M aqueous ammonia to raise the pH.

**Table S10.** Summary of MS data for deferitazole–metal complexes

MS study	$\text{Mg}^{2+}$		$\text{Mn}^{2+}$		$\text{Pb}^{2+}$		$\text{Co}^{2+}$		$\text{Ni}^{2+}$		$\text{Cu}^{2+}$		$\text{Zn}^{2+}$	
	ML	$\text{ML}_2$	ML	$\text{ML}_2$	ML	$\text{ML}_2$	ML	$\text{ML}_2$	ML	$\text{ML}_2$	ML	$\text{ML}_2$	ML	$\text{ML}_2$
Theory	421	818.2	452	849.2	605.1	1002.2	456.0	853.2	455.0	852.2	460	857.2	461.05	858.17
Observed	422.1(+)	820.9(+) 796.3(-)	452.9(+)	850.9(+) 848.9(-)	606(+)	1004.8(+)	456.9(+)	852.0(+) 854.0(-)	456.1(+)	854.9(+) 853.1(-)	459.9(+)	857.9(-)	462(+)	858.8(-)



## 6. MALDI TOF MS of Zinc(II).Deferasirox Complex

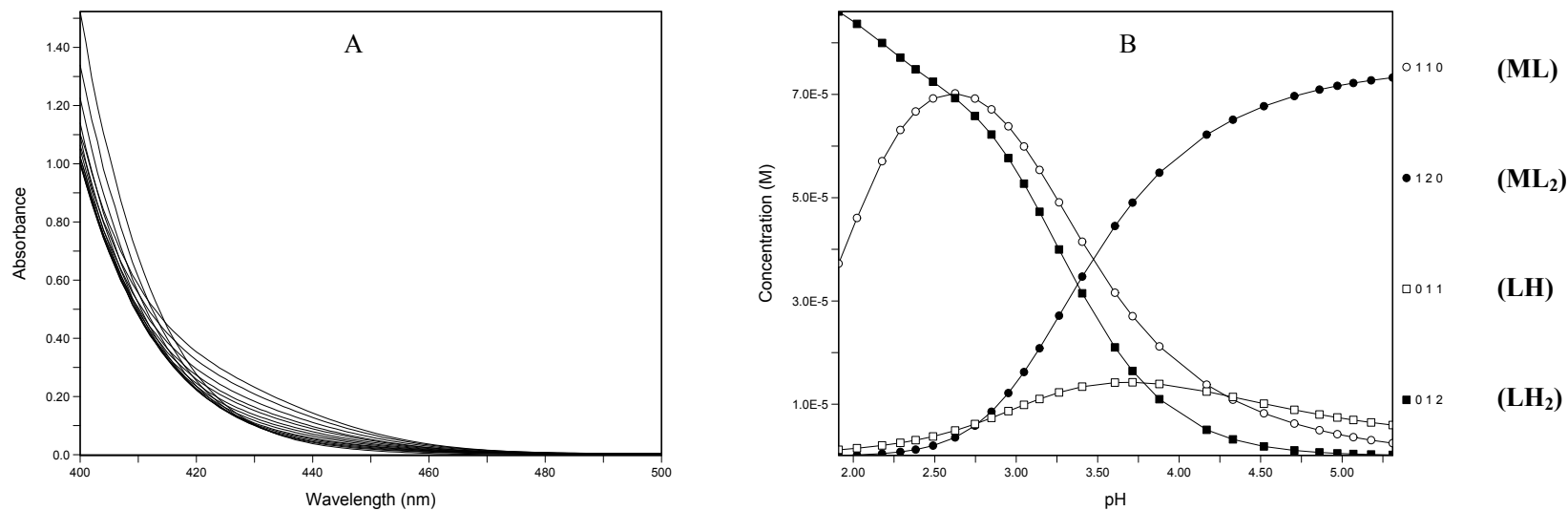


Bruker Daltonics Autoflex automated high-throughput MALDI TOF MS system with a 337 nm nitrogen laser was used.  $\alpha$ -cyano-4-hydroxycinnamic acid MALDI matrix (20 mg/mL) (0.3  $\mu$ L) was added to zinc(II).deferiasirox complex (1:1 220  $\mu$ M pH 7.4) and the mixture was allowed to dry in air. Laser energy was set to threshold power; only a single MALDI spectrum resulting from one laser shot was acquired at each location. In total, the MALDI spectra from 30 laser shots were accumulated.

**Figure S12.** Zinc(II).deferiasirox complex (1:1, 220  $\mu$ M pH 7.4) by MALDI TOF MS with  $\alpha$ -cyano-4-hydroxycinnamic acid as matrices in linear mode.

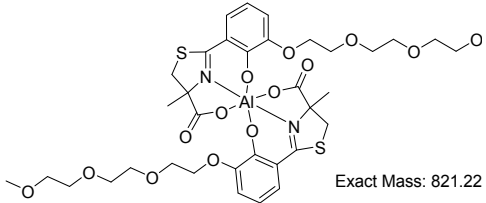
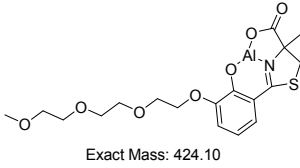
## 7. Determination of log $K$ Values for Trivalent Metals: $\text{Al}^{3+}$ and $\text{La}^{3+}$

### Log $K$ for $\text{Al}^{3+}$

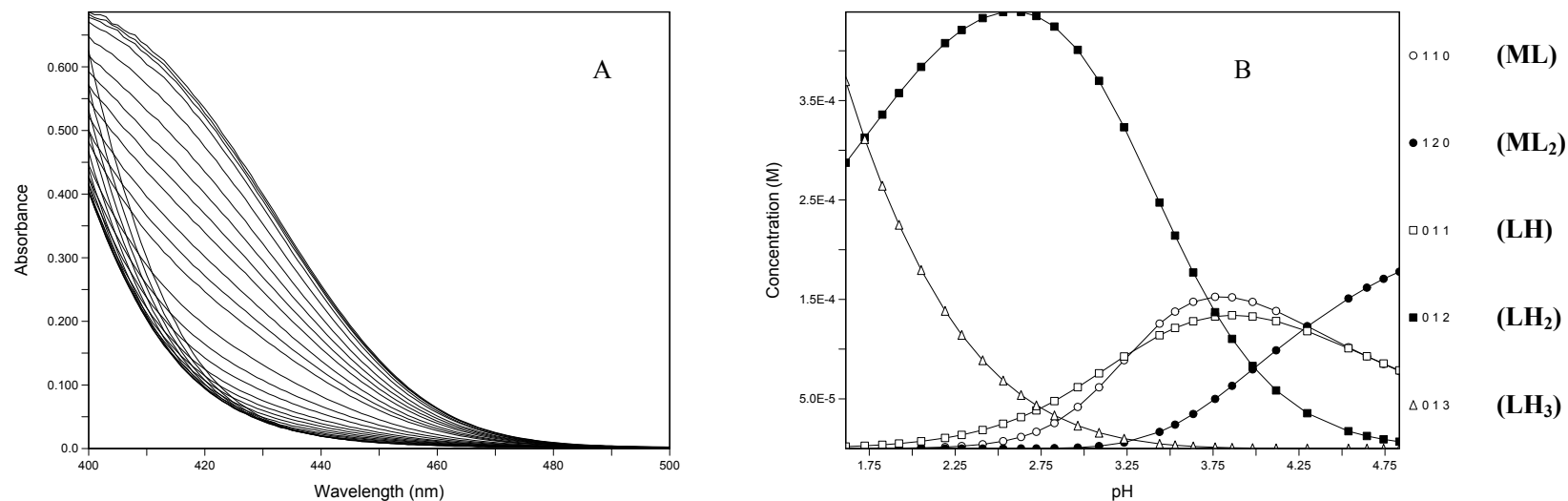


**Figure S13 (A).** Experiment with  $[\text{L}] = 180.1 \mu\text{M}$   $[\text{Al}^{3+}] = 87.9 \text{ M}$ ; ratio of L:M = 2; start in 20.153 mL 0.1 M potassium chloride at 25 °C. pH from 1.910 to 10.956; total points 57. (B) Speciation plot corresponding to conditions in (A).

**Table S11.** Structures of Al(III).deferitazole complexes, MS data and calculated affinity constants

ML <sub>2</sub>	ML	Mass spectrometry	Affinity (log)
 <p data-bbox="521 579 674 595">Exact Mass: 821.22</p>	 <p data-bbox="770 571 909 587">Exact Mass: 424.10</p>	<p data-bbox="1137 435 1330 464">Theory: 424, 821</p> <p data-bbox="1016 496 1451 587">Observed: 424.1(+); 823.1(+); 821.1(-) )</p>	<p data-bbox="1592 464 1823 496"><math>K_1 = 14.587 \pm 0.041</math></p> <p data-bbox="1592 512 1823 547"><math>\beta_2 = 26.678 \pm 0.080</math></p>

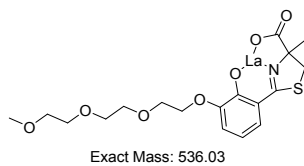
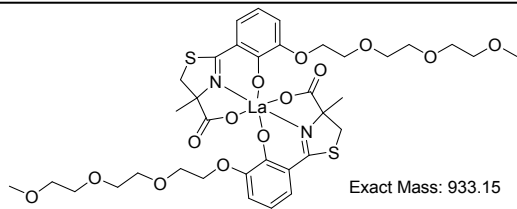
### Log *K* for La<sup>3+</sup>



**Figure S14 (A).** Experiment with [deferitazole] = 658.9  $\mu\text{M}$ ; [La<sup>3+</sup>] = 330.2  $\mu\text{M}$ ; ratio of L:M = 2; start in 24.401 mL 0.1 M potassium chloride at 25 °C. pH from 1.613 to 0.963; total points 58. (B). Speciation plot corresponding to conditions in (A).

**Table S12.** Structures of La(III).deferitazole complexes, MS data and calculated affinity constants

ML <sub>2</sub>	ML	Mass spectrometry	Affinity (log)
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Theory: 536; 933  
Observed: 935.0(+); 933.1(-)  
(ML not observed)

$$K_1 = 11.221 \pm 0.063$$
$$\beta_2 = 21.550 \pm 0.074$$