Metal Interactions in Chromatography

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Abstract

While metal-analyte interactions in IMAC (immobilized metal affinity chromatography) are widely used to analyze and purify certain compounds, the same interactions can have a detrimental effect on peak shape in other modes of chromatography. This effect has been known for a number of decades and led to the development of high purity silica in the 1980s. However, there are other sources of metal within the chromatographic system, which can cause deterioration of peak shape and sensitivity.

Coordination between the metal ion and the analyte is facilitated by lone electron pair on the analyte molecule. If two electron donar groups (either oxygen or nitrogen) are located in a favorable position, a chelate can be formed and while the enthalpy of the complex formation for two monodentate ligands and a bidentate ligand is similar, the chelate is entropically favored and leads to a stronger interaction. For this reason molecules like quinizarin, tetracycline or ciclopirox form tailing peaks in the presence of metal in the column/system.

We look at a number of ways to eliminate all sources of metal from the flow path of the HPLC system, such as tubing, frits and column body; and also investigate several functional groups on the analyte molecule responsible for the metal chelating interaction. Finally, we demonstrate the benefit of eliminating metal interactions on the quality of the separation.

Chemical Basis of Non-Specific Metal Interaction

Coordination binding between metals and organic molecules is based on the interaction between a electron donor (Lewis base) and an electron acceptor (Lewis acid). This principle was established by G.N. Lewis in 1923 and later modified by R.G. Pearson who categorized both acids and bases in hard, soft and borderline (see Table 1).

	LEWIS ACID	LEWIS BASE
HARD	H^+ Na^+ K^+ Be^{2+} Mg^{2+} Ca^{2+} Mo^{4+} Mn^{2+} Al^{3+} Sc^{3+} In^{3+} Cr^{3+} Co^{3+} Fe^{3+} Ti^{4+} Zr^{4+} U^{4+} Ce^{3+} Sn^{4+} BF_3 $AlCl_3$ AlH_3 SO_3 NO_2^+ CO_2	H_2O HO^- F^- $H_3C\text{-}CO\text{-}O^-$ PO_4^{3-} SO_4^{2-} Cl^- CO_3^{2-} ClO_4^- NO_3^- R-OH R_2O H_2N R-NH_2 $H_2N\text{-}NH_2$
BORDERLINE	Fe^{2+} Co^{2+} Ni^{2+} Cu^{2+} Zn^{2+} Pb^{2+} Sn^{2+} Sb^{3+} Bi^{3+} Ir^{3+} $B(CH_3)_3$ SO_2 Ru^{2+} R_3C^+ $C_6H_5^+$	$C_6H_5\text{-}NH_2$ C_5H_5N N_3^- Br^- NO_2^- SO_3^- N_2
SOFT	Cu^+ Ag^+ As^+ Tl^+ Hg_2^{2+} Pd^{2+} Cd^{2+} Pt^{2+} Hg^{2+} Tl^{3+} BH_3 I^+ Br^+ $H\text{-}O^+$ $R\text{-}S^+$ $R\text{-}Se^+$ I_2 Br_2 H_2C R_2C $C_6H_5(NO_2)_3$	R_2S R-SH R-S$^-$ I^- SCN$^-$ R_3P R_3As $(H_3CO)_3P$ $N\equiv C^-$ C=O $H_2C{=}CH_2$ C_6H_6 H^- R^-

Table 1: Pearson's Hard/Soft Classification of Lewis Acids and Bases

References: 1) Lewis, G. N. and Merle Randall (1923) Thermodynamics and the Free Energies of Chemical Substances. McGraw-Hill. 2) R.G.Pearson, J.Am.Chem.Soc., 85, 3533-3543, 1963

The interaction between Lewis acids and Lewis bases is especially strong when strong acids interact with strong bases and week acids interact with weak bases.

Metal Chelates

Chelates are formed when the organic partner contains two or more Lewis bases in a favorable position. The resulting 5- or 6-ring structures (Fig. 1) are entropically favored to comparable monodentate complexes. Classical examples of chelates are EDTA (Fig. 2) and imino diacetic acid.

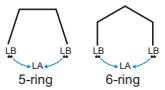

Figure 1: Stable chelate conformations

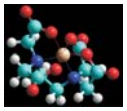

Figure 2: EDTA -Fe complex

Sources of Metal in Chromatography

Any surface that comes in contact with the sample has the potential to interact with the sample components. Since the surface areas involved are rather small (compared to the surface area of the stationary phase) these interactions manifest themselves in the form of asymmetries of the elution peak.

Metal interaction can occur on the stainless steel of the transfer tubing, the frit and the column body. Stainless steel contains ~60 % Fe, 20 % Ni and 20 % Cr, making it predominantly hard Lewis acids.

Another source of metal can be trace impurities in the silica (Fe, Ca, Al, Mg and Ti). With modern silicas the purity of the matrix improved dramatically but because the surface area is several orders of magnitude larger than the other system components the effect of impurities can still be significant.

ProteCol™ Column Hardware

In order to minimize non-specific interactions the inner walls of the column tubing is coated by either glass or PEEK™ and a porous PEEK™ frit is employed and thus all metal contact is eliminated from the flowpath. (See Fig. 3)

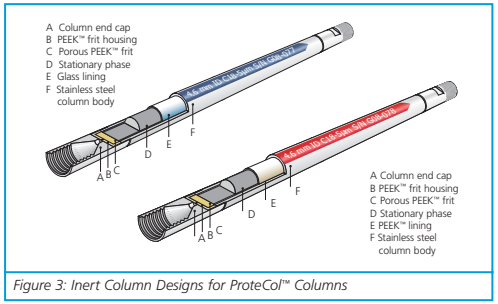

Figure 3: Inert Column Designs for ProteCol™ Columns

NIST SRM870

The NIST SRM870 testmixcontainsfive compounds (Fig. 4) and is designed to provide maximum information about the properties of the stationary phase with one simple test.

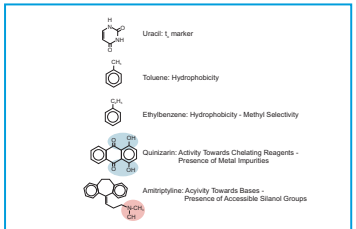

Figure 4: Test components in the NIST SRM870 test mix

NIST Results and Comparison

The column was tested with the following conditions:

Sample:	Uracil (28 µg/g) Toluene (1400 µg/g) Ethylbenzene (1700 µg/g) Quinizarin (94 µg/g) Amitriptyline (2800 µg/g) in methanol
Column:	ProteCol™-P C18 HQ105 250 mm x 4.6 mm ID
Mobile Phase:	4 mm phosphate pH7.0 in 80 % methanol
Flow rate:	1.0 mL/min
Injection volume:	1 µL
Detection:	254 nm
Temperature:	23 °C
LC system:	Shimadzu Prominence 20 AC

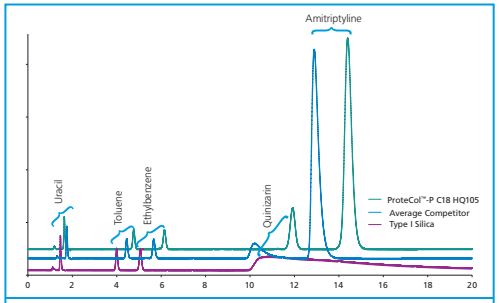

Figure 5: Chromatograms of the NIST SRM870 test mix on a variety of columns

Figure 5 shows the chromatogram achieved under the described conditions in comparison with a competitor's column and a column packed with type I silica.

In the test mix uracil is a non-retained compound and marks t_0, a value needed for further calculations. The capacity factors (k') for toluene and/or ethylbenzene allow the estimation of the hydrophobicity of the column while the relative retention between ethylbenzene and toluene gives an estimate of the selectivity of the column. The symmetry of the quinizarin and amitriptyline peaks highlight the amount of non-specific binding to metal and silanol groups respectively.

The chromatogram on the ProteCol™-P C18 HQ105 column shows symmetrical elution profiles for both quinizarin and amitriptyline indicating that there are no non-specific interactions with either silanol groups or metals.

Application: N-Hydroxypyridine-2-on

2-Hydroxypyridine-N-on is the metal chelating part of the cyclopirox molecule, an anti-fungal drug. 2-Hydroxypyridine-N-on exhibits some very strong metal affinity and is therefore a useful probe molecule to investigate non-specific metal binding. In this experiment, a stainless steel column with metal frit and a PEEK™-lined column with porous PEEK™ frits were packed with the same packing material. The columns were attached to the HPLC system with stainless steel capillaries or with PEEK™ coated fused silica (PEEKsil™).

Chromatographic conditions:

Sample:	0.1 mg/mL 2-hydroxypyridine-N-on	Mobile phase:	50 % Acetonitrile/water with 5 mm EDTA added
Injection volume:	1 µL	Flow rate:	1.0 mL/min
Column:	150 x 4.6 mm ProteCol™ C18 HQ105	Temperature:	25 °C
		Detection:	254 nm

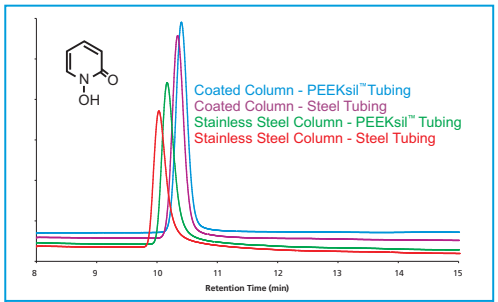

Figure 6: Effect of exposed metal in the flow path on the chromatography of chelating compounds

Results displayed as Figure 6 highlight the importance of excluding any exposed metal of getting in contact with the sample - connection capillaries, frits, column body and low activity stationary phase.

Application: Tetracycline Antibiotics

Tetracycline is a member of a group of antibiotic drugs commonly used in human and veterinary medicine. The molecule has three potential chelating sites for iron aligned at one site. The drugs are known to bind metal ions as dietary calcium and iron can render them ineffective.

Chromatographic conditions:

Sample:	1 mg/mL tetracycline (base degraded)	Gradient:	0 min - 20 % B 15 min - 40 % B 30 min - 100 % B 35 min - 100 % B 36 min - 20 % B
Injection volume:	1 µL		
Column:	250 x 4.6 mm ProteCol™ C18 HQ105 (250 x4.6 mm Exsil-ODS as Type I silica column)	Flow rate:	1.0 mL/min
		Temp.:	25 °C
		Detection:	350 nm
Mobile phase:	A: water; B: 80 % acetonitrile		

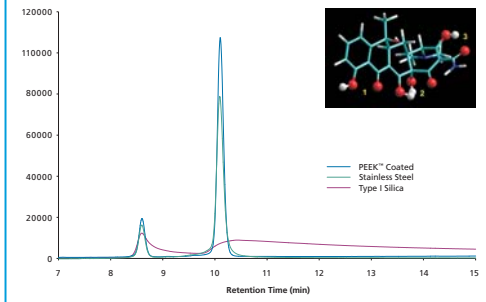

Figure 7: Chromatogram of tetracycline and its major degraduation product

Figure 7 demonstrates that changing from stainless steel column to a PEEK™ coated column increase the sensitivity (peak height) of the tetracycline peak by 35 %. There is also noticeably less peak broadening on the base of the peak. Inset: the tetracycline molecule depicting the three potential chelating groups.

Conclusions

Non-specific metal interactions anywhere in the system can have a negative effect on chromatographic performance. The extent of adverse effects are related to the amount of metal surfaces present and the strength of the interaction between the analyte and the metal surfaces. By using a combination of high purity silica, non-metallic connection capillaries and a metal-free column design it is possible to suppress non-specific interactions and significantly gain in sensitivity in the analysis of chelating samples.

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