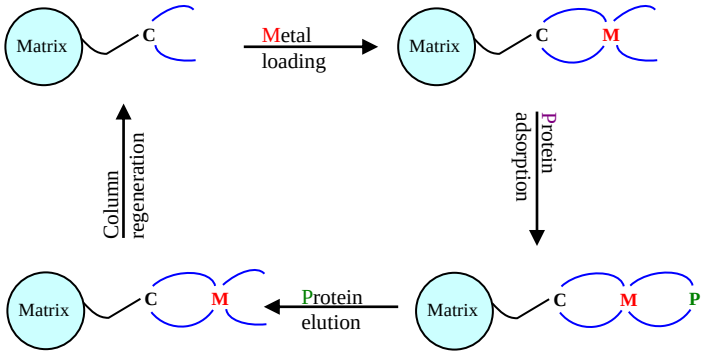
OraChrom, Inc.
The Vanguard of Liquid Chromatography.

10-B Henshaw Street Woburn, MA 01801 USA
Phone (781) 932 0151 Fax (781) 932 0787
E-mail: ***info@orachrom.com*** ***www.orachrom.com***

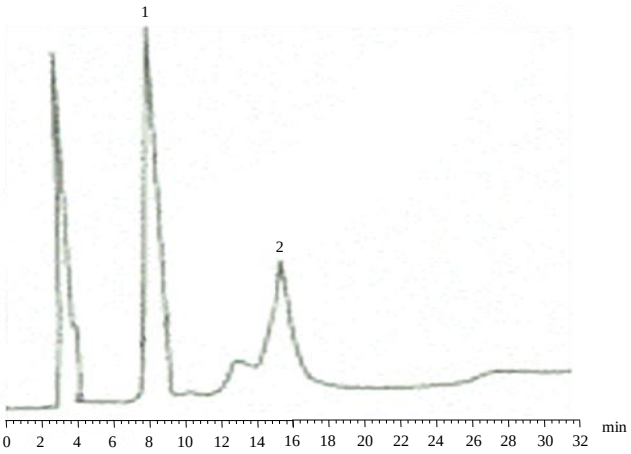
APPLICATION NOTE

Metal Chelate Liquid Chromatography on Hard Gel Gigaporous Polymeric Media: Comparison with Soft Gel.

Metal chelate Liquid chromatography includes three major steps of: a- metal loading, b- protein adsorption and c- gradient or step-wise elution of the adsorbed proteins.

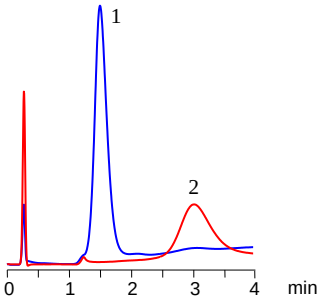


Due to flow and pressure restrictions, a typical soft gel metal chelate media would require hours of equilibration to be ready for an actual run of 20 to 30 minutes.

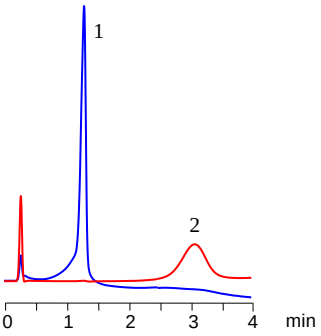


Chromatogram 1
Separation of ribonuclease A (1), from apo-transferrin (2) by a commercial soft gel metal chelate column (4.6 X 50 mm) loaded with Cu^{++}. Flow rate 0.7 ml/min (250 cm/hr)

The same separation can be performed on a hard gel gigaporous polymeric **STYROS**™ **MC-IDA** or **MC-TED** in much shorter time without compromising the resolution.



Chromatogram 2
STYROS™ MC-IDA/XP 4.6 X 50 mm loaded with Cu^{++}



Chromatogram 3
STYROS™ MC-TED/XP 4.6 X 50 mm loaded with Cu^{++}

Table 1. Operating parameters for chromatograms 2 and 3.

HPLC System.	HP 1100
Columns	As indicated
Mobile Phase	A: 20 mM Sodium Phosphate, 1 M NaCl, pH = 7.5 B: 20 mM Sodium Phosphate, 1 M NH4Cl, pH = 7.5
Flow rate	2.5 ml/min (900 cm/hr)
Gradient	0 to 100% B in 12 Column Volume
Temperature	30°C
Detection	280 nm
Injection volume	20 µl
Sample: (5 mg/ml each)	1: Ribonuclease A (bovine) 2: apo-Transferrin (bovine) (dissolved in 50 % buffer A) Proteins are assessed by the supplier to be 99% pure.

A typical **STYROS**™ **MC-IDA** column loaded with Cu^{++} can be used in as many as 50 separation cycles before it requires any regeneration.