Peptide Separations by Cation Exchange Chromatography using Luna SCX

Scott Waite, and Michael McGinley
Phenomenex Inc., Torrance, CA, USA

Introduction

While reversed phase chromatography is the traditional choice for peptide separations, alternate separation modes can provide additional information and different selectivity. Ion exchange chromatography using either a strong cation exchange (SCX) or weak anion exchange (WAX) column is a popular alternate separation mode[1] for peptide separations.

Peptide modifications such as deamidation and acetylation are not always observed by reversed phase peptide mapping, but often can be observed using ion exchange chromatography. In the field of proteomics, two-dimensional chromatography is often used to separate highly complex peptide mixtures[2]. Such techniques employ ion exchange chromatography for the first dimension separation followed by reversed phase LC-MS/MS for peptide identification.

> ***"Peptides that differ by one charge are easily separated by this method. In addition... demonstrates an ablility to separate peptides with only slight differences in hydrophobicity."***

The separation mechanism of ion exchange chromatography is based on charge differences between peptides. The sample is loaded in a low ionic strength mobile phase and eluted by increasing the ionic strength of the mobile phase. This process is usually performed via a gradient of increasing salt. Other parameters, such as pH and organic solvent, are used to modify the retentivity of different compounds. In cation exchange an increase in pH decreases the net positive charge of peptides leading to weaker binding and shorter retention times. Organic modifiers (such as acetonitrile or methanol) also affect the retentitivity of peptides by reducing hydrophobic interactions.

In this application note peptide separations were performed using Luna SCX (100Å pore silica-based column). A peptide mixture and tryptic digests of a protein were used to demonstrate the capabilities of this phase.

Instrumentation & Equipment

Analyses were performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, in-line degasser, multi-wavelength detector, and autosampler. HP Chemstation software (Version A.09.01) was used for the data analysis. The HPLC column used for the analysis was a Luna 5µ SCX 150 x 4.6mm (Phenomenex, Torrance, CA, USA). Bovine cytochrome *c* and bradykinin peptides were obtained from Sigma Chemical (St. Louis, MO, USA). Modified sequencing grade trypsin was obtained from Roche Applied Science (Indianapolis, IN, USA). HPLC grade acetonitrile, water, and trifluroacetic acid were obtained from Fisher Scientific (Springfield, NJ, USA).

Figure 1. Peptides Separations with Luna SCX

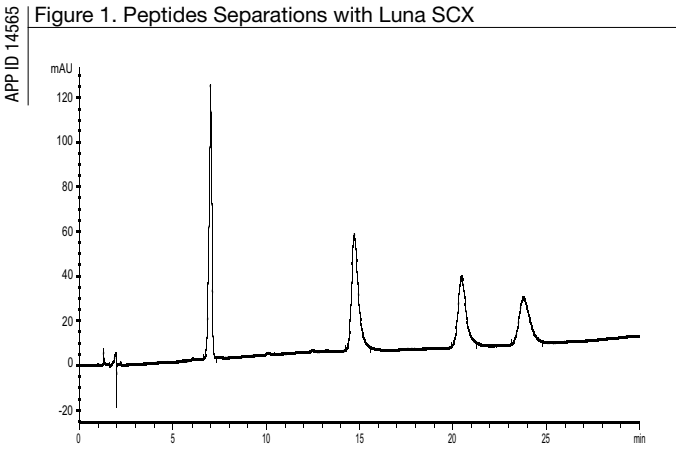

Figure 1: Ion exchange chromatogram of a peptide mixture. The mixture is of four different bradykinin peptides: bradykinin fragment 2-7, bradykinin fragment 1-7, Ile-Ser bradykinin, and full-length bradykinin. Peptides vary between 0 to +2 net charge at running conditions (pH 2.5). More positively charged peptides elute later in the chromatogram with increased salt concentrations.

Experimental Method

Bradykinin Peptide Mixture

A mixture of bradykinin peptides was reconstituted in water and diluted with mobile phase A prior to injection. The composition of mobile phase A was 20 mM potassium phosphate pH 2.5: acetonitrile (75:25). Mobile phase B used for the peptide mixture was 20 mM potassium phosphate pH 2.5/0.5 M potassium chloride:acetonitrile (75:25). The column was equilibrated with 100% A prior to sample injection. The gradient program consisted of a linear ramp from 0% B to 100% B over 30 minutes and the column was re-equilibrated with 100% A prior to subsequent injections.

Cytochrome *c* Tryptic Digests

Tryptic digests were generated as follows: a 100 µg aliquot of cytochrome *c* was dissolved in 200 µL of 0.1 M Ammonium bicarbonate pH 8.0. A 4 µL aliquot of 0.5 mg/mL trypsin was added (1:50 E/S ratio) and incubated at 37°C for 18 hours. Reaction was stopped by acidification with 4 µL of trifluroacetic acid and sample was diluted with water prior to injection on HPLC. An aliquot of 50 µL (20 µg) of the cytochrome *c* digest was injected for analysis by peptide mapping.

For peptide mapping similar HPLC conditions to the peptide separations were used except mobile phase B was 20 mM potassium phosphate pH 2.5/350 mM potassium chloride: acetonitrile (75:25).

Figure 2. Ion Exchange (SCX) Peptide Map of Cytochrome *c*

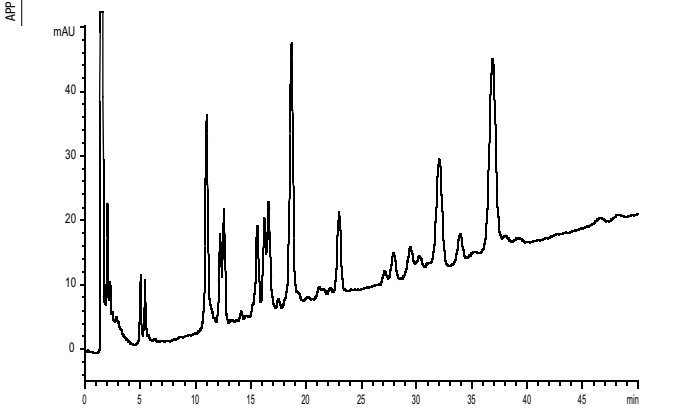

Figure 2: Ion exchange chromatograph of a tryptic digest of cytochrome *c*. Approximately 20 µg of the digest was injected on the Luna SCX 150 x 4.6 column. Note sharp peak shape and resolution of peptides.

Also, the gradient program used was a linear ramp from 0% B to 100% B over 50 minutes. For all analyses column temperature was maintained at 35°C and the flow rate was 1.0 mL/min throughout the run. Elution of sample was monitored by UV at 215 nm.

Results and Discussion

Luna SCX is a silica-based ion exchange media with a phenyl sulfonic acid group as the reactive ligand. The presence of phenyl group conveys some reversed phase characteristics to the media and thus acetonitrile can be used in the mobile phase to improve peak shape. HPLC analyses of a mixture of bradykinin peptides demonstrate the ability of Luna SCX to separate peptides based on charge differences (figure 1). Peptides that differ by one charge are easily separated by this method. In addition, separation of similarly charged peptides (i.e. Ile-Ser bradykinin and full-length bradykinin) demonstrates an ablility to separate peptides with only slight differences in hydrophobicity.

An example of using ion exchange chromatography for peptide mapping is shown in figure 2. Numerous peaks are well resolved with excellent peak symmetry. Tryptic digestion of cytochrome *c* using the described digestion conditions typically generates between 15-20 peptides. Many of these peptides are cationic with similar isoelectric points; other ion exchange media frequently demonstrate incomplete resolution of these peptides with poor peak shapes. These chromatograms demonstrate the ability of the Luna SCX media to resolve various peptide mixtures.

References

1. F. Regnier and R. Chicz in *HPLC of Biological Macromolecules* K.Gooding and F. Regneir (Ed.) pg. 77-93 (1990) Marcel Dekker

2. M.Ronk et. al. in *Techniques in Protein Chemistry V* J.Crabb (Ed.) pg.259-268 (1994) Acedemic Press

Ordering Information

Description	Order No.
Luna 5µ SCX 100Å 50 x 4.6mm	00B-4398-E0-TN
Luna 5µ SCX 100Å 150 x 4.6mm	00F-4398-E0-TN
Luna 5µ SCX 100Å 250 x 4.6mm	00G-4398-E0-TN
SecurityGuard Cartridges 10/pk**	AJ0-4308

* Other sizes available, contact Phenomenex for more details.

** SecurityGuard Cartridges require universal holder Order No.: KJ0-4282

3678_L

For a digital copy of this Technical Note, please visit www.Phenomenex.com/TechNotes/1024