

Influencing Selectivity in LC-MS Peptides Analysis on the Raptor ARC-18 Column

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Abstract

Methods for LC-MS peptide analysis often use acidified mobile phases, which can alter retention and selectivity. Here, we demonstrate the effects of modifying acid type, acid concentration, temperature, and gradient slope using several test probes. A Raptor ARC-18 column was employed for this work because the stationary phase ligand is sterically protected, making it extremely stable and resistant to acid damage at low pHs, which is an important characteristic when developing LC-MS methods for peptide analysis.

Introduction

With the influx of biotherapeutics in medical research and healthcare, LC-MS analysis of peptides and small proteins continues to grow. Many of these analyses utilize acid-modified mobile phases to improve peak shape [1]; however, the effects of acidic mobile phases on selectivity and retention are often not well understood. In this article, we will explore the effects of acid type, acid concentration, temperature, and gradient slope on the selectivity and retention of several peptide probes and one small protein probe (cytochrome C) using the Raptor ARC-18 LC column. This column was selected because the ligand is sterically protected from acid damage and is stable to pH 1.0. In addition, it contains superficially porous particles (SPP), which allow for faster analysis times than can be achieved with fully porous particle (FPP) columns.

Figure 1: Raptor ARC-18 LC Column Properties.

Column Description			
	Stationary Phase Category: C18, octadecylsilane (L1)	Recommended Usage: pH Range: 1.0-8.0 Maximum Temperature: 80 °C Maximum Pressure: 600 bar / 8,700 psi (2.7 µm) or 400 bar / 5,800 psi (5 µm)	
	Ligand Type: Sterically protected C18	Particle: 2.7 µm or 5 µm superficially porous silica (SPP or "core-shell")	Properties: <ul style="list-style-type: none"> • Well-balanced retention profile. • Sterically protected to resist harsh, low-pH mobile phases. • Ideal for use with sensitive detectors like mass spec.
	Pore Size: 90 Å	Surface Area: 150 m ² /g (2.7 µm) or 100 m ² /g (5 µm)	

Experimental

Several experiments were carried out to demonstrate the effects of acid-modified mobile phases, temperature, and gradient slope on LC-MS peptide analysis. For this work, the peptide and small protein standards in Table I and a bovine serum albumin (BSA) trypsin digest standard (Protea) were diluted in acidified water. These standards were injected into a UHPLC system configured with a PDA and QDa for simultaneous UV and MS detection. A wavelength of 215 nm and electrospray ionization in positive ion mode was used for UV and MS detection, respectively. Reversed-phase separations were performed using water and acetonitrile mobile phases modified with 0.01, 0.05, and 0.1% formic acid (FA) or trifluoroacetic acid (TFA) under gradient conditions using a Restek Raptor ARC-18 LC column. Specific conditions for each experiment are presented in Figures 2-5.

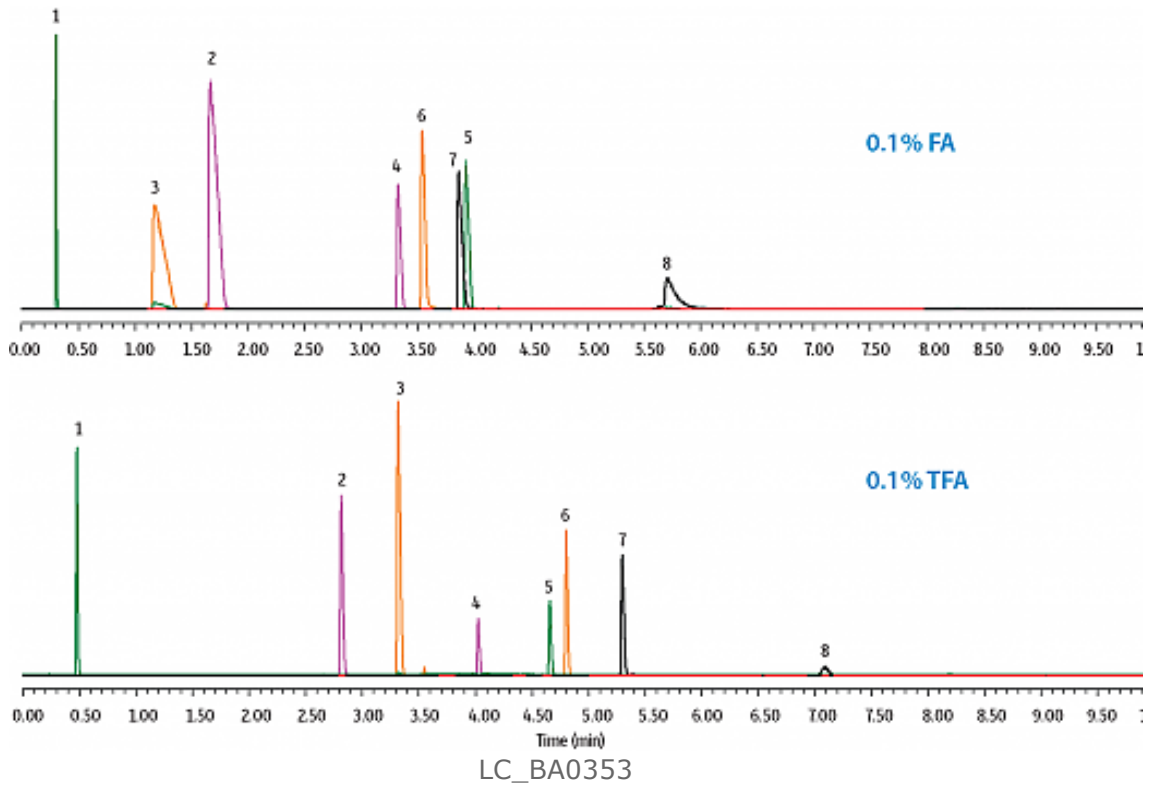
Table I: Probes used for LC-MS Peptide Analyses

Peak ID	Compound	Molecular Weight (Da)	SIR (m/z)
1	GLY-TYR	238	239.3
2	VAL-TYR-VAL	380	380.5
3	MET-ARG-PHE-ALA	524	524.6
4	Methionine enkephalin	574	574.6
5	Leucine enkephalin	556	556.7
6	Angiotensin II	1,046	524.4
7	Neurotensin	1,700	837.9
8	Cytochrome C	12,300	1,020.6

Results and Discussion

Several probes were used to aid in the understanding of selectivity changes that affect LC-MS peptide analyses when the type and concentration of the acidic modifier in the mobile phase is manipulated. The retention of peptides containing basic amino acids (e.g., arginine) exhibited considerably large changes in retention when switching between formic acid and TFA. In Figure 2, the peptide MET-ARG-PHE-ALA not only changed elution order but also displayed improved peak shape and was more retained due to the increased ion pairing capabilities of TFA over formic acid. Alternatively, VAL-TYR-VAL is a polar peptide with an overall neutral charge whose changes in selectivity result from the favorable decrease in mobile phase pH when TFA is used; in this case, the peptide was less ionized and therefore better retained. Variations in acid concentration had a comparable effect. In Figure 3, overall retention times increased with increasing concentrations of TFA, and improvements in peak shape were observed for the small protein, cytochrome C (12.3 kDa). For this reason, sterically protected columns that are stable at low pH, such as the Raptor ARC-18 column, are often preferred for LC-MS peptide analysis.

Figure 2: Effects of Acid Type on Selectivity and Peak Shape.



Column Raptor ARC-18 (cat.# 9314A12)
 Dimensions: 100 mm x 2.1 mm ID
 Particle Size: 2.7 μ m
 Pore Size: 90 \AA

Temp.: 60 $^{\circ}$ C

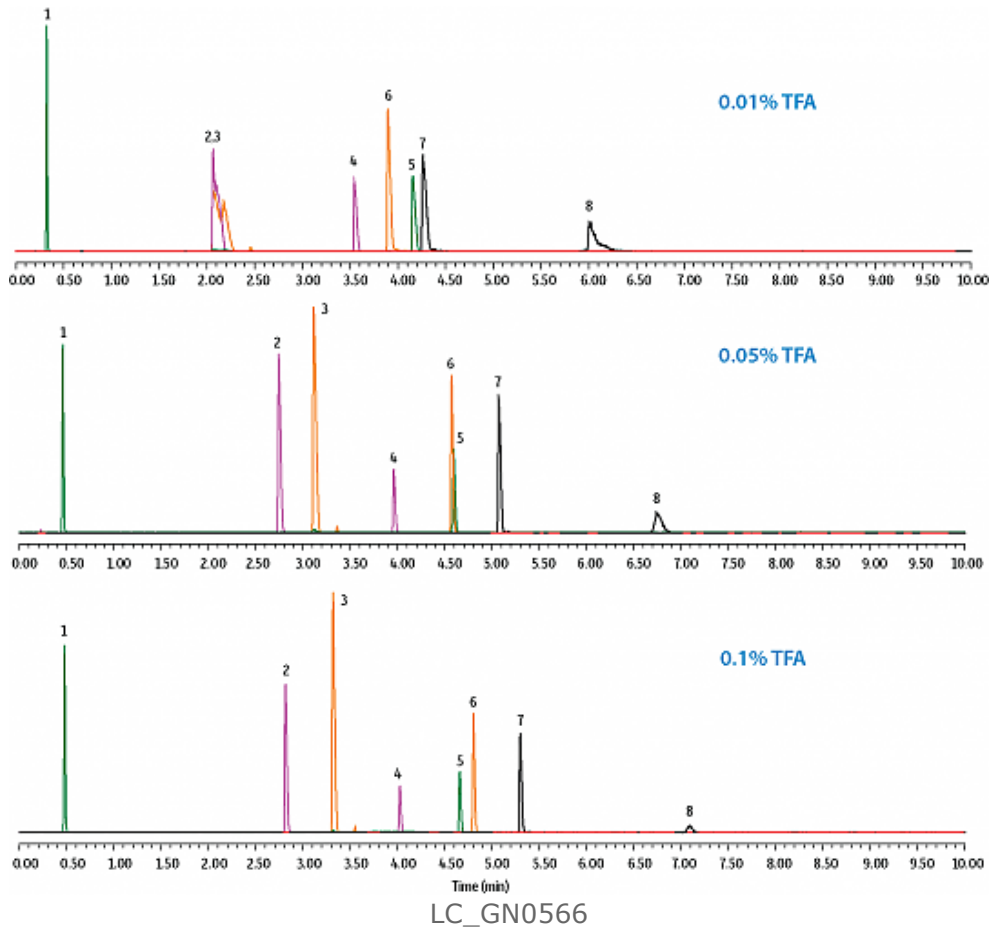
Sample Custom peptide/small protein standard mix
 Diluent: 0.1% Formic acid in water
 Conc.: 0.2–0.4 mg/mL
 Inj. Vol.: 2 μ L

Mobile Phase
 A: Water + acid
 B: Acetonitrile + acid

Time (min)	Flow (mL/min)	%A	%B
0.00	1	95	5
1.00	1	95	5
10.0	1	50	50
10.1	1	95	5
13.0	1	95	5

Detector MS
 Ion Mode: ESI+
 Mode: SRM
Instrument UHPLC

Figure 3: Effects of Acid Concentration on Retention and Selectivity.



Column Raptor ARC-18 (cat.# 9314A12)
 Dimensions: 100 mm x 2.1 mm ID
 Particle Size: 2.7 μm
 Pore Size: 90 \AA

Temp.: 60 $^{\circ}\text{C}$

Sample Custom peptide/small protein standard mix
 Diluent: Water + 0.1% formic acid
 Conc.: 0.2–0.4 mg/mL
 Inj. Vol.: 2 μL

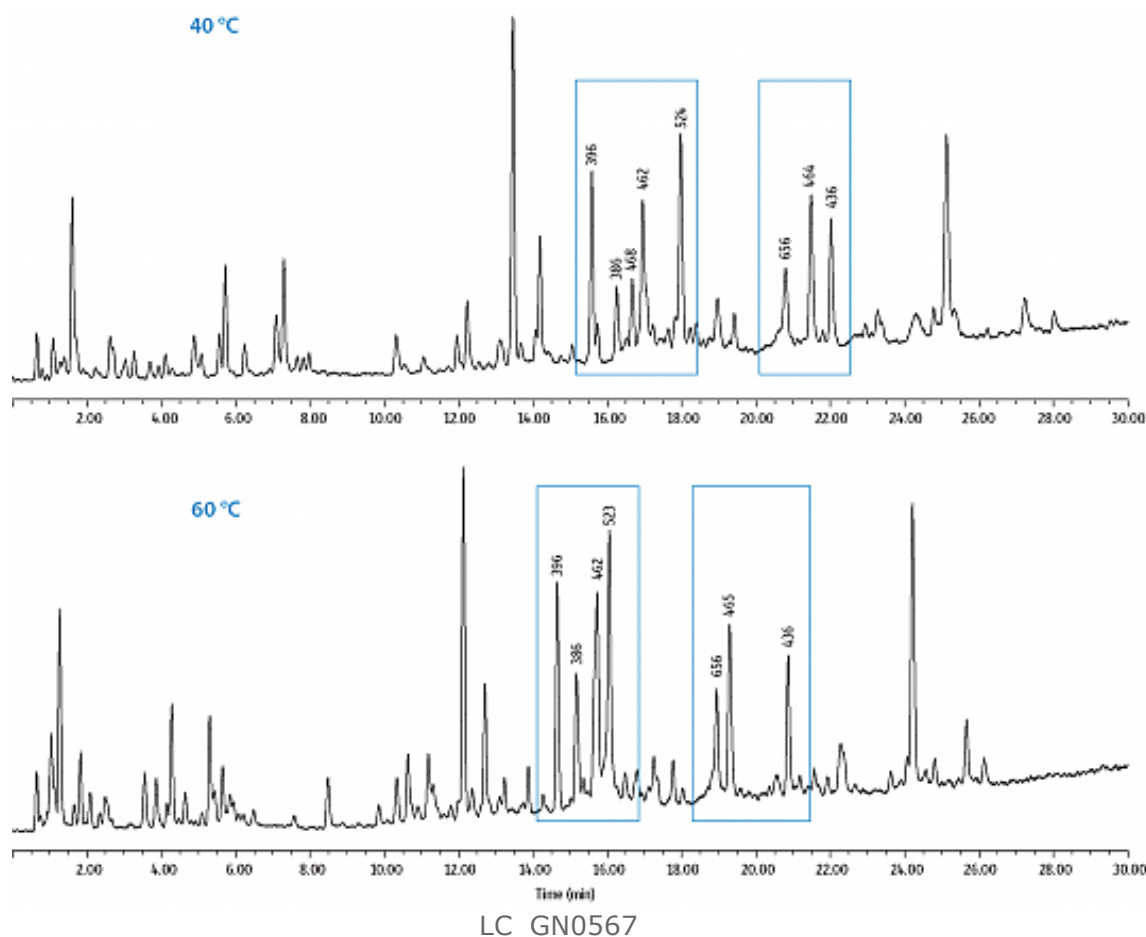
Mobile Phase
 A: Water + TFA
 B: Acetonitrile + TFA

Time (min)	Flow (mL/min)	%A	%B
0.00	1	95	5
1.00	1	95	5
10.0	1	50	50
10.1	1	95	5
13.0	1	95	5

Detector MS
 Ion Mode: ESI+
 Mode: MS scan
Instrument UHPLC

In a second set of experiments, resolution and elution order changes were examined when temperature and gradient modifications were made to the analysis of a bovine serum albumin (BSA) trypsin digest standard. In the resulting peptide maps shown in Figure 4, base peak mass 468 m/z was resolved from an adjacent peak when a lower column oven temperature (40 °C) was used. Similarly, the gradient slope was shown to impact the band spacing of closely eluting compounds. This is demonstrated in Figure 5 where the number of resolved peaks increased with decreasing gradient slope. In general, steeper gradients can be used for the separation of specific peptides in order to achieve short cycle times. However, if numerous peptides from a digest are to be analyzed, a shallower gradient is typically preferred.

Figure 4: Effects of Temperature on the Selectivity of Peptides in a BSA Tryptic Digest Standard.



Column Raptor ARC-18 (cat.# 9314A12)
 Dimensions: 100 mm x 2.1 mm ID
 Particle Size: 2.7 µm
 Pore Size: 90 Å

Sample Bovine serum albumin (BSA) tryptic digest standard
 Diluent: Water + 0.1% formic acid
 Conc.: 5 pmol
 Inj. Vol.: 2 µL

Mobile Phase

A: Water + 0.1% formic acid

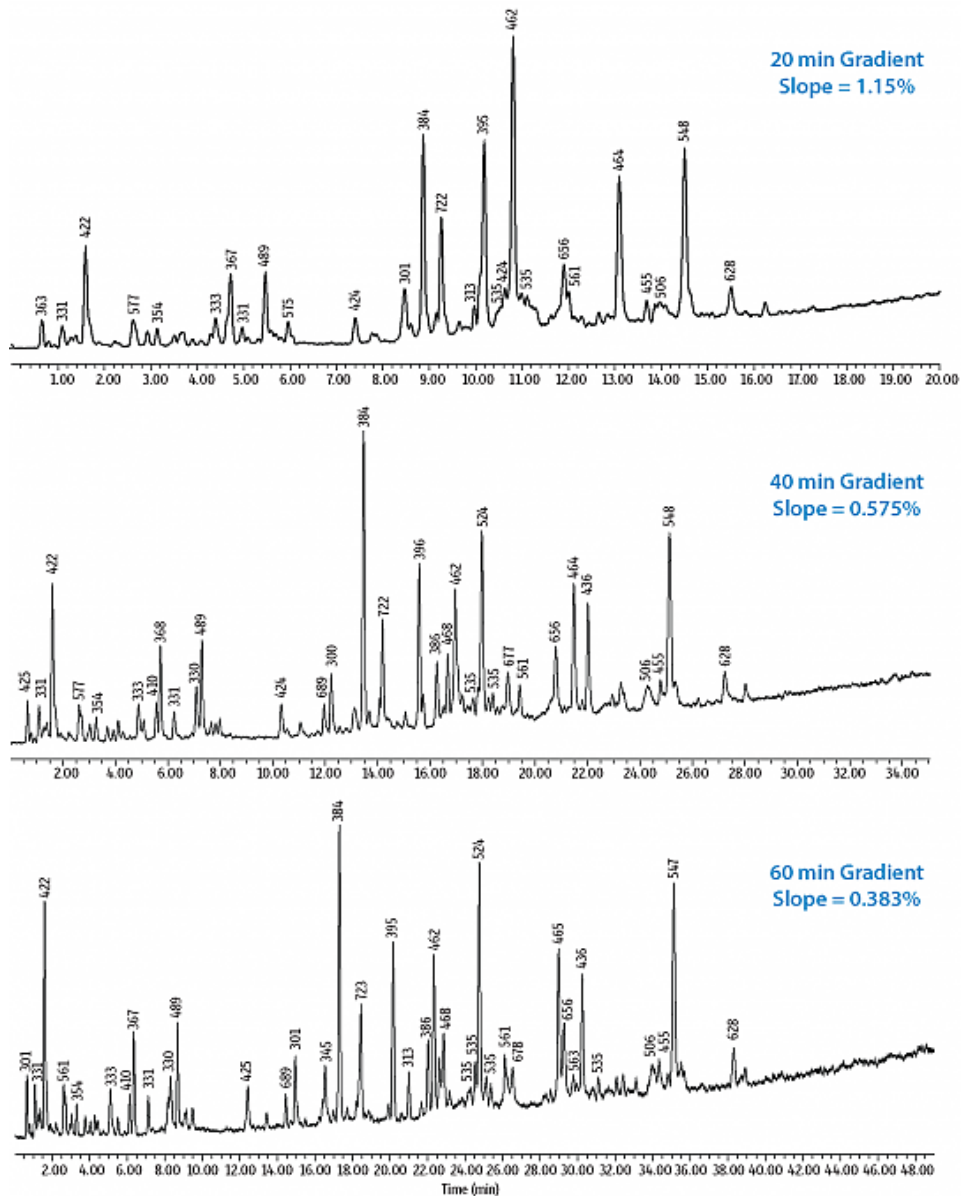
B: Acetonitrile + 0.1% formic acid

Time (min) Flow (mL/min) %A %B

Time (min)	Flow (mL/min)	%A	%B
0.00	0.3	98	2
40.0	0.3	75	25
40.1	0.3	98	2
50.0	0.3	98	2

Detector MS
Ion Mode: ESI+
Mode: MS scan
Instrument UHPLC

Figure 5: Effects of Gradient Steepness on the Selectivity of Peptides in a BSA Tryptic Digest Standard.



Column	Raptor ARC-18 (cat.# 9314A12)
Dimensions:	100 mm x 2.1 mm ID
Particle Size:	2.7 μm
Pore Size:	90 \AA
Temp.:	40 $^{\circ}\text{C}$
Sample	Bovine serum albumin (BSA) tryptic digest standard
Diluent:	Water + 0.1% formic acid
Conc.:	5 pmol
Inj. Vol.:	2 μL
Mobile Phase	
A:	Water + 0.1% formic acid
B:	Acetonitrile + 0.1% formic acid
	Flow (mL/min)%A %B
	0.3 98 2
	0.3 75 25
	0.3 98 2
	0.3 98 2
Detector	MS
Ion Mode:	ESI+
Mode:	MS scan
Instrument	UHPLC

Conclusion

Relatively small changes in chromatographic parameters can have a large impact on LC-MS peptide analysis. In this work, we demonstrated that significant shifts in selectivity and retention can occur in response to relatively small changes to mobile phase acid (type and concentration), temperature, and gradient slope. Sterically protected columns like the Raptor ARC-18 column are stable at low pH and provide the versatility required for the development of peptide applications.

References

[1] E. Wang, D. Kalu Appulage, F. Carroll, K. Schug, Intact protein separation and quantitation in biological fluids using HPLC and triple quadrupole mass spectrometry, ASMS, St. Louis, MO, 2015.