

# A Novel Screening Approach for Comparing LC-MS Reversed-Phase and HILIC Methods for Separations in Biological Matrices Using Amino Acid Examples

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## Introduction

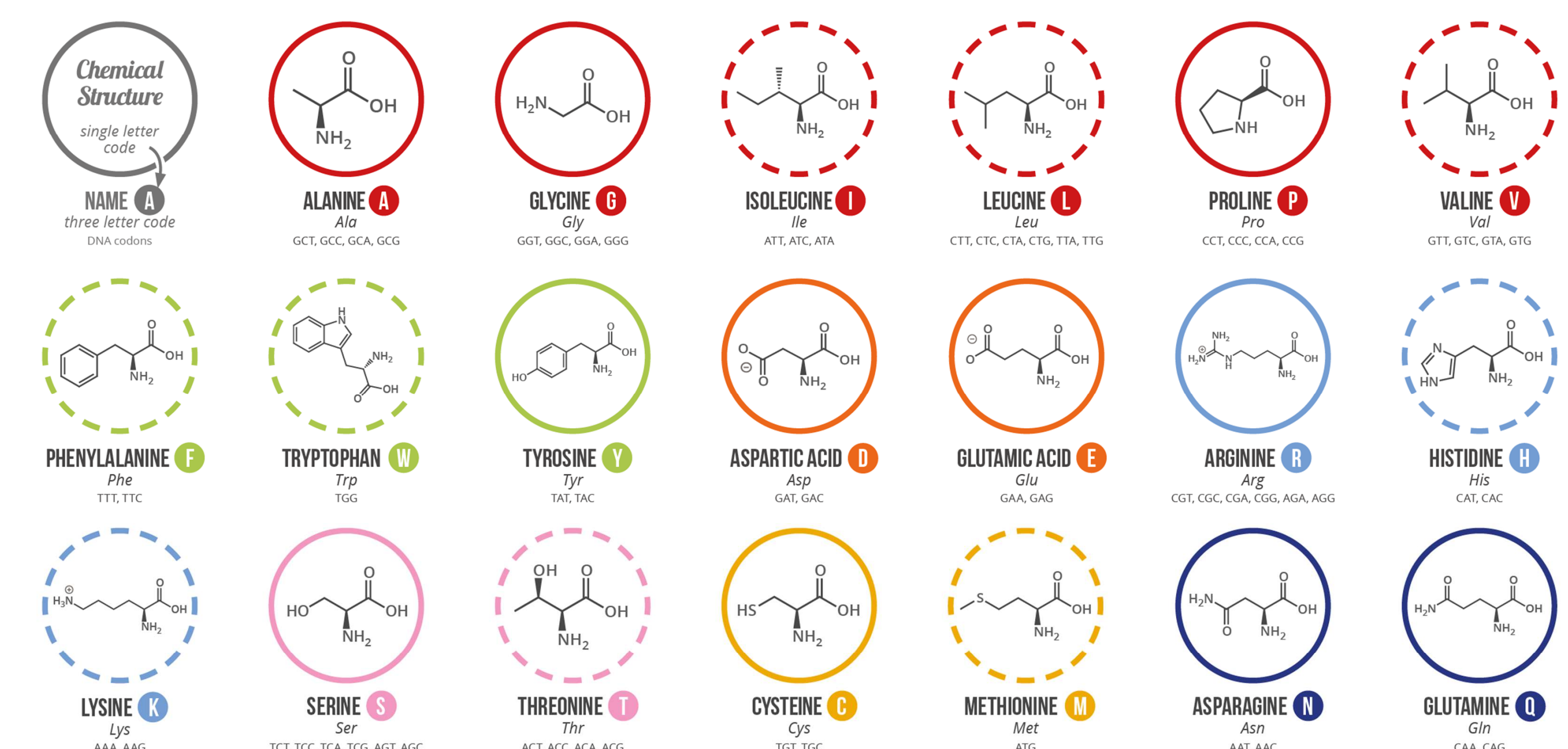
Metabolites are often studied via liquid chromatography-mass spectrometry (LC-MS) in both reversed-phase (RPLC) and hydrophilic interaction (HILIC) modes. Yeast extract and heat-deactivated human serum were utilized as model biological systems to demonstrate the orthogonal selectivity achievable with the use of both HILIC and RPLC separations in the analysis of amino acids. A novel gradient technique was employed to save time and to avoid the need to prepare new mobile phases when RP columns are exchanged for HILIC columns. Generating LC-MS comparisons with RP or HILIC columns can be done with the same mobile phase reservoirs while changing only gradient profiles to suit RP or HILIC mode.

## Amino Acids Overview

### A GUIDE TO THE TWENTY COMMON AMINO ACIDS

AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. ESSENTIAL AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILE NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESIZED IN THE BODY.

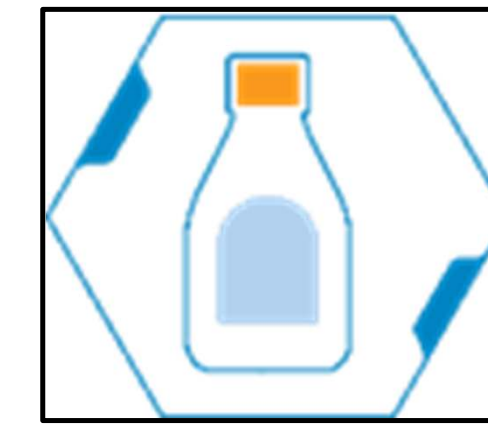
**Chart Key:** ● ALIPHATIC ● AROMATIC ● ACIDIC ● BASIC ● HYDROXYLIC ● SULFUR-CONTAINING ● AMIDIC ○ NON-ESSENTIAL ○ ESSENTIAL



**Note:** This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

**Figure 1:** A guide to the twenty common amino acids. Amino acid metabolism is involved in a myriad of metabolic mechanisms, including tissue growth, energy production, immune function, and nutrient absorption. Isomers such as leucine and isoleucine can be challenging to separate via RPLC since they are often completely unretained under even slightly organic elution conditions. (as shown in figure 2)

## HPLC Method Conditions: RP and HILIC



A: 8 mM Ammonium Formate, pH 3.0 (aq.), in 100% water

### Reversed Phase Method:

Column: HALO 90 Å AQ-C18, 2.7µm 2.1 x 150mm

Part Number: 92812-722

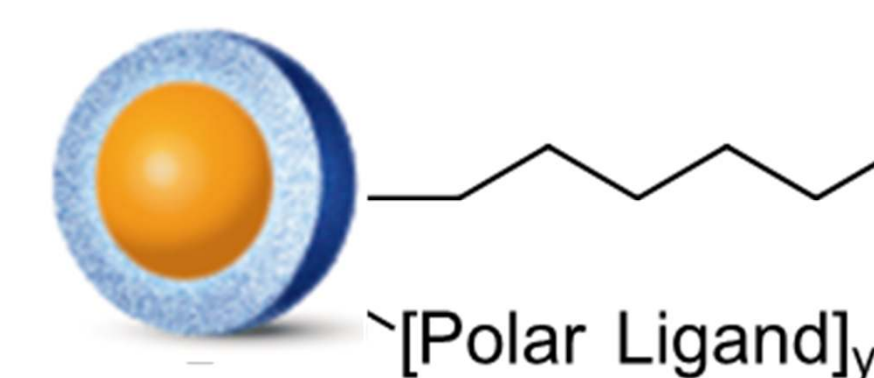
Gradient:	Time	%B
	0.0	0
	3.0	0
	18.0	100
	21.0	100

Flow Rate: 0.5 mL/min

Temperature: 35 °C

Detection: LC/MS QExactive HF Hybrid Orbitrap

Injection Volume: 2 µL



### HILIC Method:

Column: HALO 90 Å Penta-HILIC, 2.7µm 2.1 x 150mm

Part Number: 92812-705

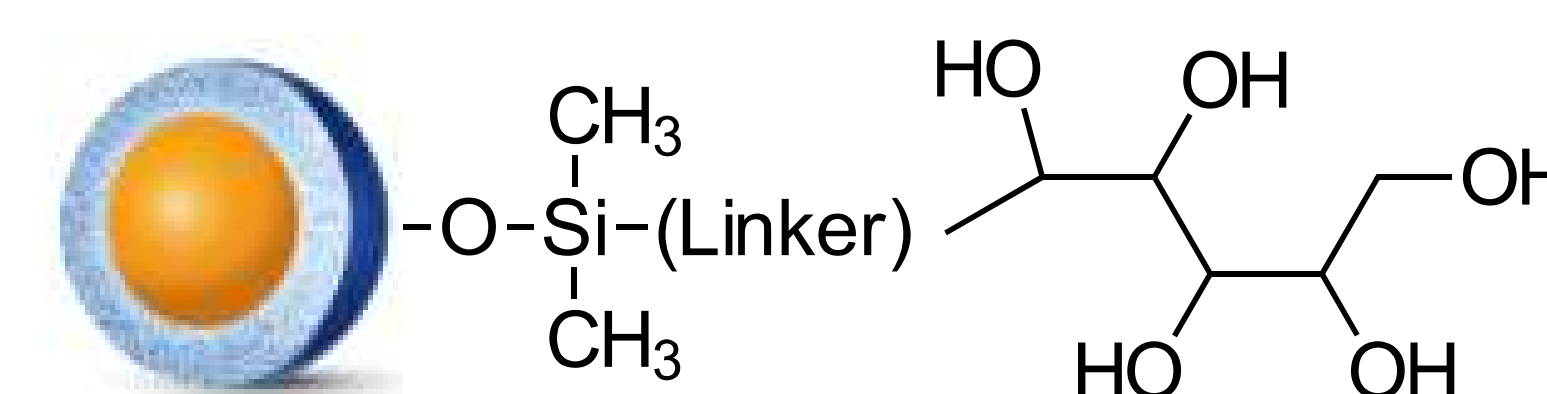
Gradient:	Time	%B
	0.0	100
	2.0	100
	17.0	53
	20.0	53

Flow Rate: 0.3 mL/min

Temperature: 35 °C

Detection: LC/MS QExactive HF Hybrid Orbitrap

Injection Volume: 2 µL



### MS Conditions:

System: ThermoFisher QExactive

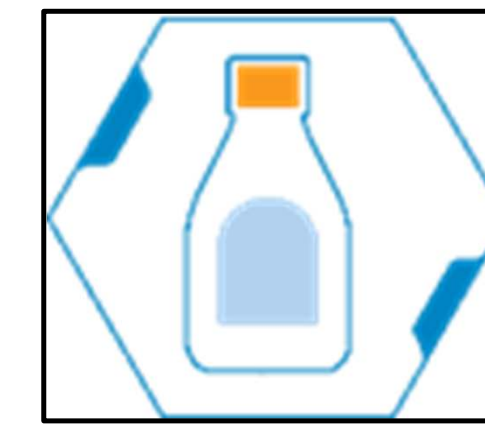
HF Hybrid Orbitrap

Spray Voltage (kV): 3.5

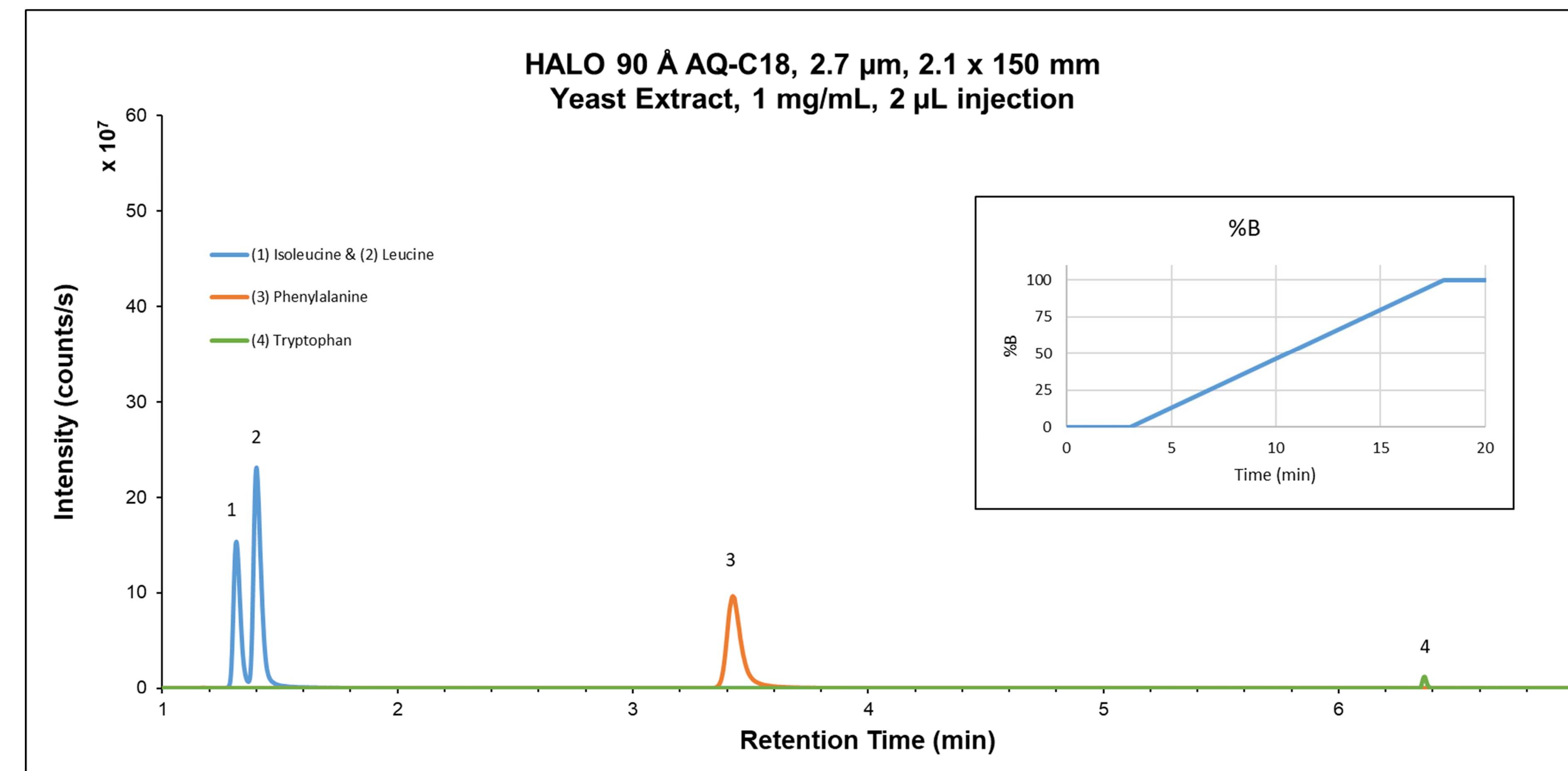
Capillary Temperature: 350 °C

Sheath Gas: 60 (RP) 20 (HILIC)

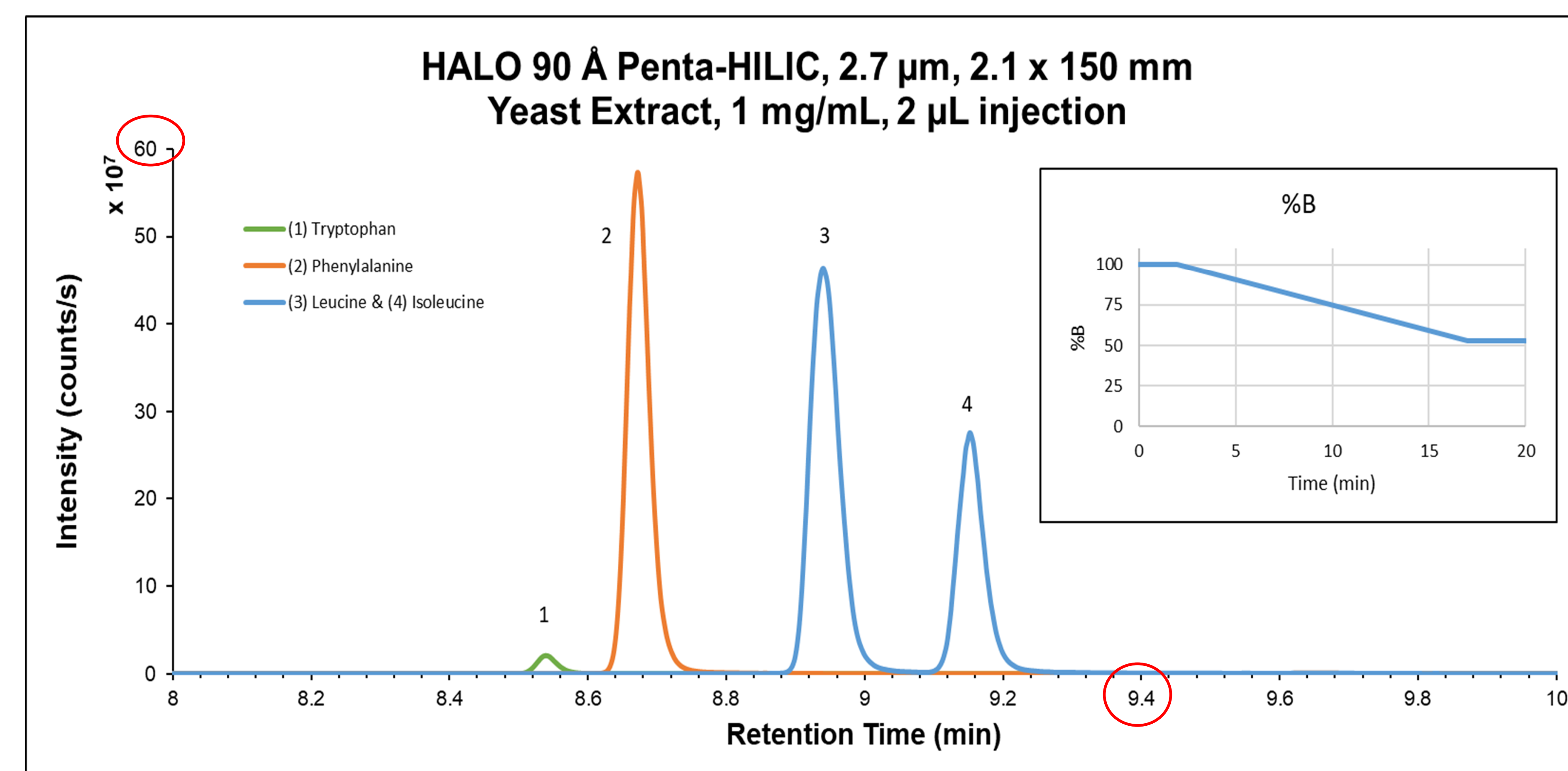
RF Lens: 40



B: 8 mM Ammonium Formate, pH 3.0 (aq.), in 95:5 acetonitrile: water

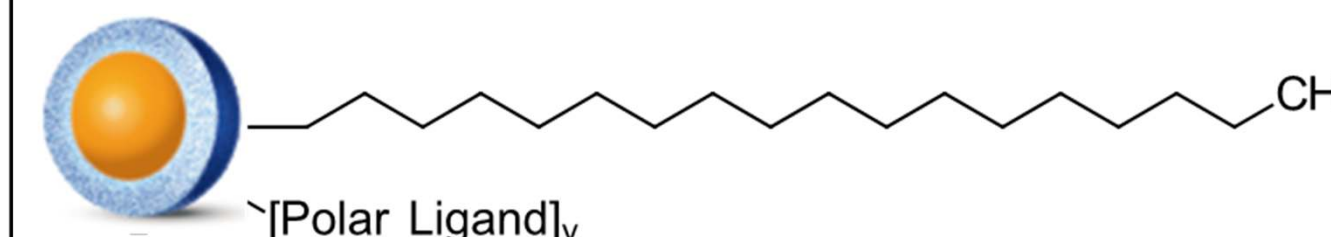
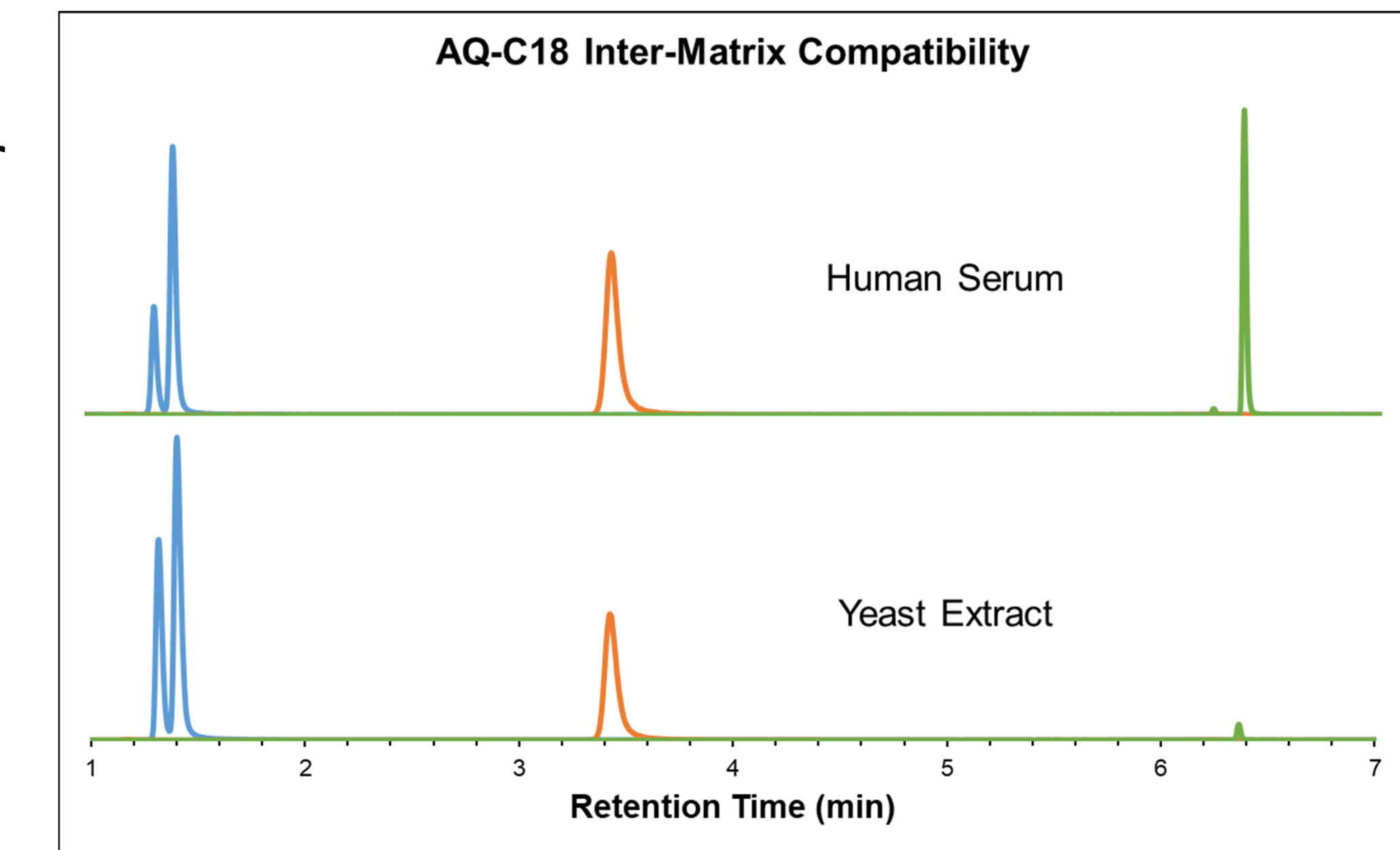


**Figure 2:** Reversed Phase Amino Acid Analysis: HALO® AQ-C18

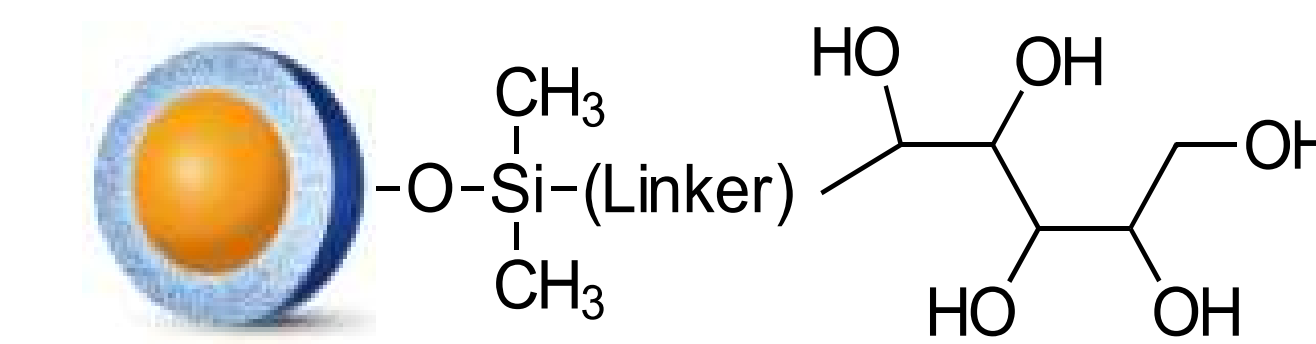


**Figure 3:** HILIC Amino Acid Analysis: HALO® Penta-HILIC

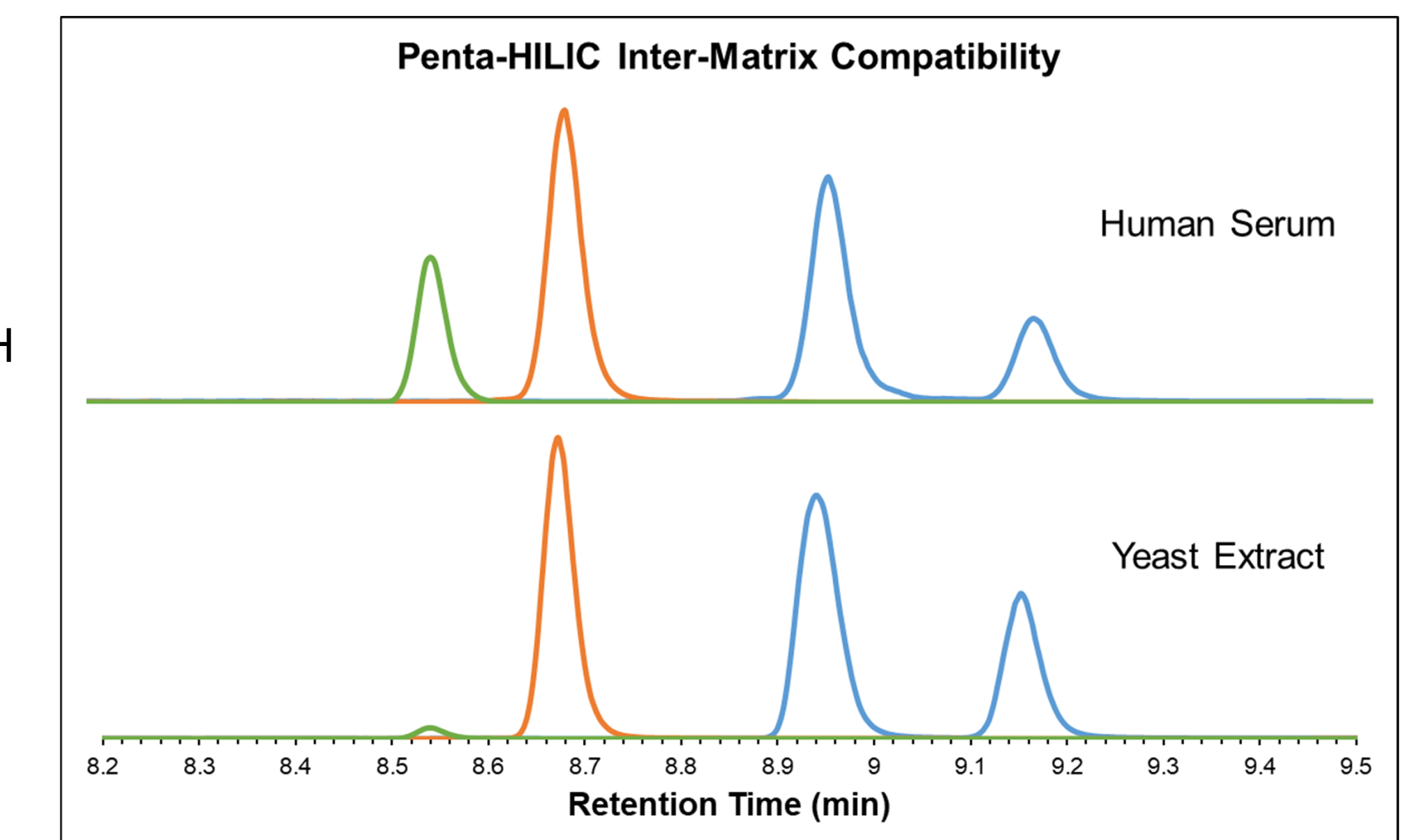
## Inter-Matrix Compatibility



**Figure 4:** AQ-C18 Inter-Matrix Compatibility



**Figure 5:** Penta-HILIC Inter-Matrix Compatibility



It is important to ensure that different sample matrices show similar results and repeatability. Both the HALO® AQ-C18 and Penta-HILIC stationary phases exhibit excellent inter-matrix compatibility, as demonstrated in the analysis of yeast extract and heat-deactivated human serum in **Figure 4/5**.

## Conclusions

Metabolomic analyses are utilized in various avenues of clinical research, including drug discovery, disease characterization, and pharmacodynamic evaluation. Metabolites are often studied via liquid chromatography-mass spectrometry (LC-MS) in both reversed-phase (RPLC) and hydrophilic interaction (HILIC) modes. A novel gradient technique was employed to save time and to avoid the need to prepare new mobile phases when RP columns are exchanged for HILIC columns. Generating LC-MS comparisons with RP or HILIC columns can be done with the same mobile phase reservoirs while changing only gradient profiles to suit RP or HILIC mode. This screening technique can be easily automated and expanded by using column switching valves.