

# APPLICATIONS

## Reproducible LC-MS/MS Separation of 38 Amino Acids in HILIC Mode using a bioZen™ Glycan LC Column

Zuzana Demianova<sup>1</sup>, Jeff Layne<sup>2</sup>, Brian Rivera<sup>2</sup>, Elsa Gorre<sup>3</sup>, Andrew Mahan<sup>3</sup> and Lei Xiong<sup>4</sup>

<sup>1</sup>SCIEX, Brea, CA, USA; <sup>2</sup>Phenomenex, Torrance, CA, USA;

<sup>3</sup>Janssen Research & Development, Spring House, PA, USA;

<sup>4</sup>SCIEX, Redwood Shore, CA, USA

### Introduction

Amino acids are the building blocks of proteins, making them an extremely well researched compound class. With thousands of naturally occurring amino acids available, separating and quantitating a mixture of amino acids can be challenging. While HPLC is a well characterized method for amino acid analysis, LC-MS analysis of amino acids poses several challenges: 1. Some compounds are very polar and are hard to retain by reversed phase HPLC, 2. Isomers can coelute and are difficult to differentiate from each other, and 3. While effective, HILIC based separation sometimes results in poor reproducibility. These challenges were overcome by combining the separation power of the bioZen Glycan HILIC phase LC column with SWATH® Acquisition, resulting in highly reproducible resolution and accurate quantitation and confirmation of 38 amino acids.

### Materials and Methods

A sample of 38 amino acids was prepared using a standard stock mix (A9906, Sigma-Aldrich®), and used as 10x diluted for various experiments.

Buffers were prepared as follows:

Make 1 L of 100 mM Ammonium formate, then adjust pH to 3.1 with formic acid. Then, combine 900 mL of acetonitrile or water with 100 mL of the prepared buffer. Final buffer concentration should equal 10 mM in each bottle.

### HPLC Conditions

**Column:** bioZen Glycan, 2.6 µm  
**Dimensions:** 100 x 2.1 mm  
**Part No.:** [00D-4773-AN](#)  
**Elution Type:** Gradient  
**Mobile Phase:** A: 10 mM Ammonium formate in Acetonitrile  
 B: 10 mM Ammonium formate in Water

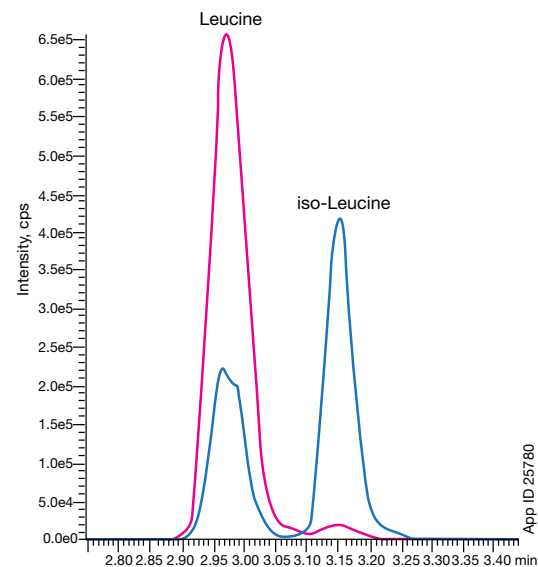
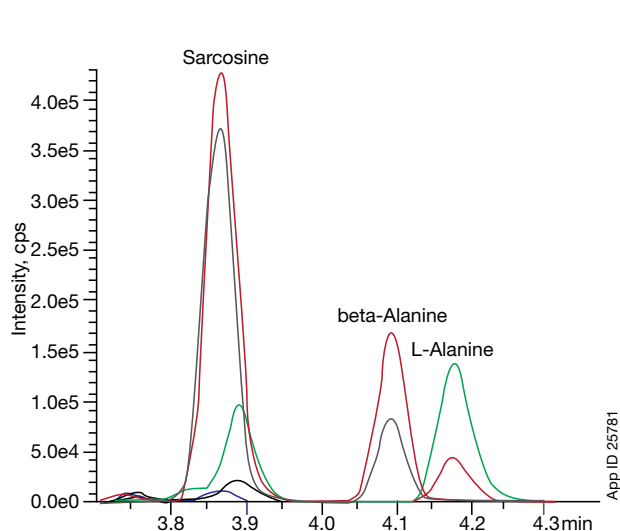
Gradient:	Time (min)	% B
	0.01	0
	2	5
	7	50
	8	50
	8.1	0
	12	0

**Flow Rate:** 500 µL/min  
**Temperature:** 40 °C  
**System:** TripleTOF® 6600 coupled with an ExionLC™ system (SCIEX)  
**MS Acquisition:** SWATH® Acquisition  
**MS Detection:** MS/MS

**Sample:**

1. Urea	20. 3-Methyl-L-histidine
2. L-Creatine	21. L-Lysine
3. 2-Aminoethanol	22. Beta-Alanine
4. L-Tryptophan	23. L-Ornithine
5. L-Phenylalanine	24. Glycine
6. L-Leucine	25. L-Threonine
7. L-Isoleucine	26. Anserine
8. Taurine	27. L-Histidine
9. L-Methionine	28. L-Alanine
10. L-Tyrosine	29. Hydroxylysine
11. L-Proline	30. L-Carnosine
12. L-Valine	31. L-Serine
13. Gamma-aminobutyric acid	32. L-Citrulline
14. L-alpha-aminoisobutyric acid	33. L-Homocystine
15. L-Sarcosine	34. L-Cystathionine
16. DL-3-aminoisobutyric acid	35. L-Cystine
17. L-Arginine	36. L-Aspartic acid
18. 1-Methyl-L-histidine	37. 2-Aminoadipic acid
19. Hydroxy-L-proline	38. L-Glutamic acid

**Figure 1.**  
 Baseline separation of isomers (SWATH MS/MS spectra)



**Results and Discussion**

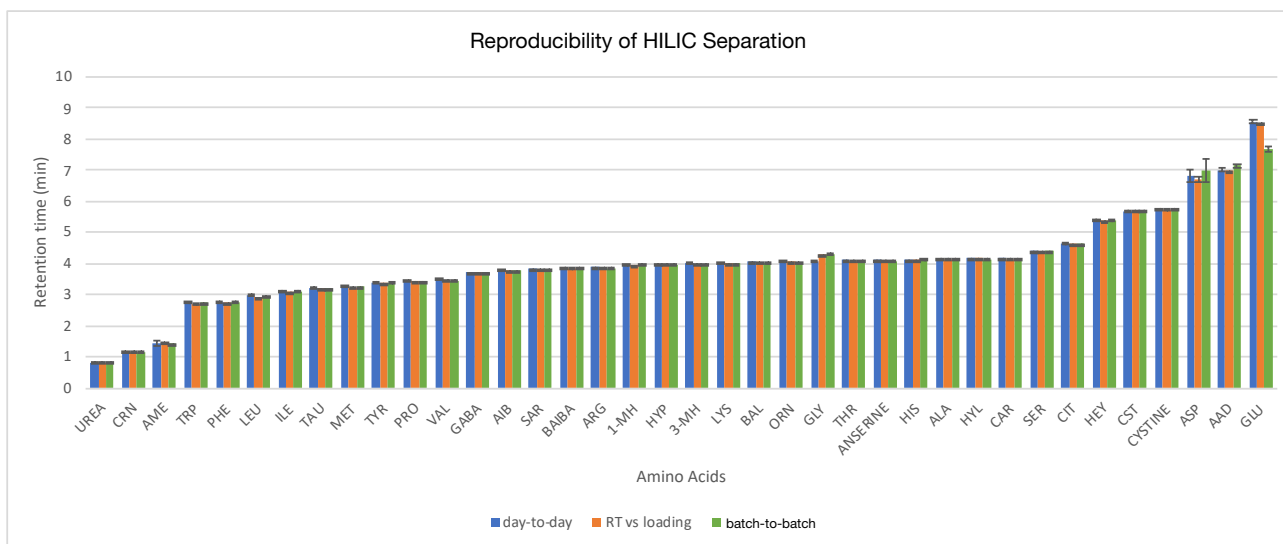
Due to the polar and hydrophilic nature of amino acids, HILIC chromatography was utilized to separate the 38 amino acid mixture. HILIC mode provided better retention and baseline isomeric separation as compared to the reversed phase separation of these compounds (**Figure 1**). Although HILIC mode was successful in separating the 38 amino acids in our analysis, it was imperative that the reproducibility of the separation be challenged. Inter-day, intra-day, lot-to-lot, and multiple injection volumes were studied to verify the robustness of our method (**Figure 2**). This was performed by measuring any fluctuation in run times under various conditions. The results of these studies are depicted in **Figure 2**, where the following conditions were studied:

1. 3 batches and 15 injections per batch
2. Day-to-day evaluation which includes 4 days and 5 injections per day
3. Run time vs. loading evaluation (1, 2, 3, 4, and 5 µL injection volumes at 3 injections per volume)

**Conclusion**

Utilizing a highly reproducible bioZen Glycan LC column (HILIC phase) and a robust LC-MS/MS method, 38 amino acids were successfully separated. This methodology was tested for robustness under various conditions including inter- and intra-day, various media lots, and several different injection sizes to ensure that the separation was not only successful but also robust and reproducible. Furthermore, this methodology using MS detection, especially SWATH® Acquisition, allows for accurate amino acid quantitation in various biological matrixes

**Figure 2.** Reproducibility study of the bioZen™ Glycan LC column (HILIC)



**Ordering Information**

bioZen Columns (mm)			Biocompatible Guard Cartridges	
Phase	50 x 2.1	100 x 2.1	for 2.1 mm	Holder
bioZen 2.6 µm Glycan	<a href="#">00B-4773-AN</a>	<a href="#">00D-4773-AN</a>	<a href="#">00F-4773-AN</a>	<a href="#">AJ0-9800</a> <a href="#">AJ0-9000</a>



**BE-HAPPY™**  
guarantee

Your happiness is our mission. Take 45 days to try our products. If you are not happy, we'll make it right.

[www.phenomenex.com/behappy](http://www.phenomenex.com/behappy)

**Australia**

t: +61 (0)2-9428-6444  
 auinfo@phenomenex.com

**Austria**

t: +43 (0)1-319-1301  
 anfrage@phenomenex.com

**Belgium**

t: +32 (0)2 503 4015 (French)  
 t: +32 (0)2 511 8666 (Dutch)  
 beinfo@phenomenex.com

**Canada**

t: +1 (800) 543-3681  
 info@phenomenex.com

**China**

t: +86 400-606-8099  
 cninfo@phenomenex.com

**Denmark**

t: +45 4824 8048  
 nordicinfo@phenomenex.com

**Finland**

t: +358 (0)9 4789 0063  
 nordicinfo@phenomenex.com

**France**

t: +33 (0)1 30 09 21 10  
 franceinfo@phenomenex.com

**Germany**

t: +49 (0)6021-58830-0  
 anfrage@phenomenex.com

**India**

t: +91 (0)40-3012 2400  
 indiainfo@phenomenex.com

**Ireland**

t: +353 (0)1 247 5405  
 eireinfo@phenomenex.com

**Italy**

t: +39 051 6327511  
 italiainfo@phenomenex.com

**Luxembourg**

t: +31 (0)30-2418700  
 nlinfo@phenomenex.com

**Mexico**

t: 01-800-844-5226  
 tecnicomx@phenomenex.com

**The Netherlands**

t: +31 (0)30-2418700  
 nlinfo@phenomenex.com

**New Zealand**

t: +64 (0)9-4780951  
 nzinfo@phenomenex.com

**Norway**

t: +47 810 02 005  
 nordicinfo@phenomenex.com

**Poland**

t: +48 (12) 881 0121  
 pl-info@phenomenex.com

**Portugal**

t: +351 221 450 488  
 ptinfo@phenomenex.com

**Singapore**

t: +65 800-852-3944  
 sginfo@phenomenex.com

**Spain**

t: +34 91-413-8613  
 espinfo@phenomenex.com

**Sweden**

t: +46 (0)8 611 6950  
 nordicinfo@phenomenex.com

**Switzerland**

t: +41 (0)61 692 20 20  
 swissinfo@phenomenex.com

**Taiwan**

t: +886 (0) 0801-49-1246  
 twinfo@phenomenex.com

**United Kingdom**

t: +44 (0)1625-501367  
 ukinfo@phenomenex.com

**USA**

t: +1 (310) 212-0555  
 info@phenomenex.com

🌐 **All other countries/regions**  
**Corporate Office USA** 🌐

t: +1 (310) 212-0555  
 info@phenomenex.com

**www.phenomenex.com**

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com

**Terms and Conditions**

Subject to Phenomenex Standard Terms and Conditions which may be viewed at [www.phenomenex.com/TermsAndConditions](http://www.phenomenex.com/TermsAndConditions).

**Trademarks**

bioZen is a trademark of Phenomenex. SWATH and TripleTOF are registered trademarks and ExionLC is a trademark of AB Sciex Pte. Ltd. AB SCIEX is being used under license. Sigma-Aldrich is a registered trademark of Sigma-Aldrich Co., LLC

FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.

© 2020 Phenomenex, Inc. All rights reserved.