AccQ•Tag SOLUTIONS

Retention Time Tables for Amino Acids Using the AccQ•Tag™ Method

In amino acid analysis, constituents of non-protein or modified protein samples often produce additional peaks to the standard chromatogram, potentially interfering with the interpretation of results. In some analyses, the amine compound of interest is not normally found in proteins. How does one determine what constitutes these additional peaks, or where an unknown amino acid elutes? This application note describes the chromatographic retention properties in an AccQ•Tag separation for a wide variety of amino acids not present in standard protein hydrolysates. The following tables illustrate the approximate retention times (RT) of diverse amine compounds under AccQ•Tag separation conditions.

The choice of reaction conditions is determined by the optimization of the separation of desired amino acids. The retention coefficients (RC) listed in the tables are the quotient of the peak's RT divided by the RT of phenylalanine.

Table 1

Compound Name and Sample Type Code		Retention Coefficient pH = 5.05
β-Hydroxyaspartic Acid	3	0.186
Galactosamine-1	2	0.206
Glucosamine-1	2	0.228
Cysteic Acid	3	0.244
Phosphoserine	3	0.259
γ-Carboxy Glutamic Acid	3	0.274
Hydroxyproline	4	0.353
Aminoquinoline	1	0.353
Glucosamine-2	2	0.386
Phosphothreonine	3	0.386
Aspartic Acid	1	0.406
Galactosamine-2	2	0.446
Serine	1	0.446
Asparagine	5	0.447
Glutamic Acid	1	0.468
Carboxymethylcysteine	3	0.468
Glycine	1	0.501
Glutamine	5	0.518
Histidine	1	0.519
Ammonia	1	0.566
Trimethyllysine	7	0.575
1-Methylhistidine	6	0.586
3-Methylhistidine	6	0.605
Dimethyllysine	7	0.610
Taurine	5	0.618
Methionine Sulphoxide-1	3	0.621
Methionine Sulphoxide-2	3	0.626
Arginine	1	0.628
Threonine	1	0.637
Sulfopropyl Cysteine	3	0.640
Cysteine, Ethyltrimethyl	3	0.640
Phosphotyrosine	3	0.646
Cysteine, Thioglycolic	3	0.649
Alanine	1	0.656
Methionine Sulphone	3	0.660
Cysteine, Propyltrimethyl	3	0.662
Methylarginine-1	7	0.664
Dimethylarginine	7	0.693
Proline	1	0.695
γ-Aminobutyric Acid	6	0.701
Methylarginine-2	7	0.708
α-Aminobutyric Acid	8	0.734
Cysteine, Thiopropionic	3	0.737
Cysteine, Propyltriethyl	3	0.757
Cystine	1	0.781
Tyrosine	1	0.791
Valine	1	0.825
Cysteine, Thiobutyric	3	0.825
Methionine	1	0.840
Norvaline	8	0.843
Ornithine	6	0.884
Lysine	1	0.913
Isoleucine	1	0.942
Leucine	1	0.960
Methyllysine	7	0.961
Norleucine	8	0.980
Nitrotyrosine	7	0.987
Phenylalanine	1	1.000
Tryptophan	5	1.034

This table indicates the RC's of the derivatives using the standard hydrolysate method (eluent pH 5.05).

Table 2

Compound Name and Sample Type Code		Retention Coefficient pH = 5.80
Galactosamine-1	2	0.183
Glucosamine-1	2	0.194
Cysteic Acid	3	0.205
Phosphoserine	3	0.205
Aspartic Acid	1	0.257
Glutamic Acid	1	0.324
Phosphothreonine	3	0.325
Hydroxyproline	4	0.336
Carboxymethylcysteine	3	0.350
Glucosamine-2	2	0.363
Serine	1	0.426
Asparagine	5	0.439
Galactosamine-2	2	0.439
Aminoquinoline	1	0.453
Glycine	1	0.478
Glutamine	5	0.511
Histidine	1	0.536
Ammonia	1	0.556
Phosphotyrosine	3	0.570
Taurine	5	0.589
Cysteine, Thioglycolic	3	0.589
Threonine	1	0.596
Arginine	1	0.613
Alanine	1	0.625
Methionine Sulphone	3	0.640
γ-Aminobutyric Acid	6	0.651
Proline	1	0.689
α-Aminobutyric Acid	8	0.724
Tyrosine	1	0.779
Cystine	1	0.793
Valine	1	0.801
Methionine	1	0.821
Hydroxylysine-1	4	0.835
Hydroxylysine-2	4	0.850
Ornithine	6	0.907
Isoleucine	1	0.907
Leucine	1	0.933
Lysine	1	0.948
Norleucine	8	0.955
Phenylalanine	1	1.000
Tryptophan	—	1.028

This table contains the RC's for the derivatives using a method run with eluent pH 5.80.

Sample type codes indicate the type of matrix in which the amino acid can be found.

Waters

Table 1 and 2 represent data from in-house and customer experiments. These tables may not include data on all amino acid separations possible with the AccQ•Tag method.

Sample Codes

Sample	Code
Hydrolysate Amino Acids	1
Glycoproteins	2
Derivatized / Modified Proteins	3
Collagen Samples	4
Non-hydrolyzed Biologic Samples	5
Physiologic Samples	6
Novel Biochemical Intermediates	7
Common Internal Standard AA's	8

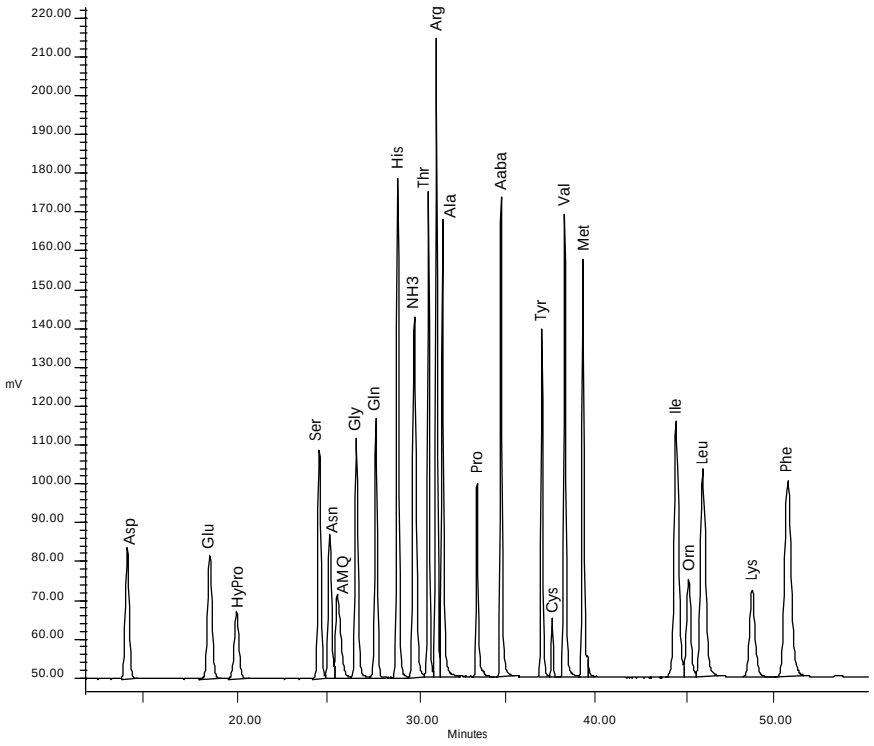
Separation Protocols

The two separation protocols described earlier by their pH values differ in a number of other key parameters including ionic strength and triethylamine content of eluents, column temperature, and gradient profile.

The net effect of these changes is that the pH 5.80 gradient, while slower (60 vs 45 min.), resolves Asn, Gln, Hypro and other non-hydrolysate derivatives. In addition, the Ser / Glu, Arg / Thr and Cys / Tyr pairs reverse their order, the AMQ reagent peak moves from first to sixth in elution order, and Lys moves from before to after the Ile / Leu pair.

Two derivatives will likely be resolved completely if the difference between their retention coefficients is greater than 0.015. All reported values should be considered approximations, which may not be identical for all system configurations.

Waters AccQ•Tag Method has been shown to provide successful separations of a wide variety of amino acids in diverse sample matrices.



Further Reading

Sensitive Analysis of Cystine / Cysteine using 6-Aminoquinolyl-H-Hydroxysuccinimidyl Carbamate (AQC) Derivatives, Techniques in Protein Chemistry IV, 1993, Academic Press.

Waters AccQ•Tag Amino Acid Analysis System Operater's Manual Number 154-02TP, Waters Corporation.

Waters

Waters Corporation
34 Maple Street
Milford, MA 01757
(508) 478-2000
FAX (508) 872-1990
http: / / www.waters.com /