Waters Alliance® LC/MS System

The Use of 6-Aminoquinolyl-N-Hydroxy Succinimidyl Carbamate Derivatives for HPLC/MS Analysis of Amino Acids

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Key Words
AccQ•Tag® reagent
Amino acid analysis
Cone voltage
Electrospray Ionization, ESI
Linearity
Positive and negative modes
Post-column derivatization
Sensitivity
ZMD mass detector

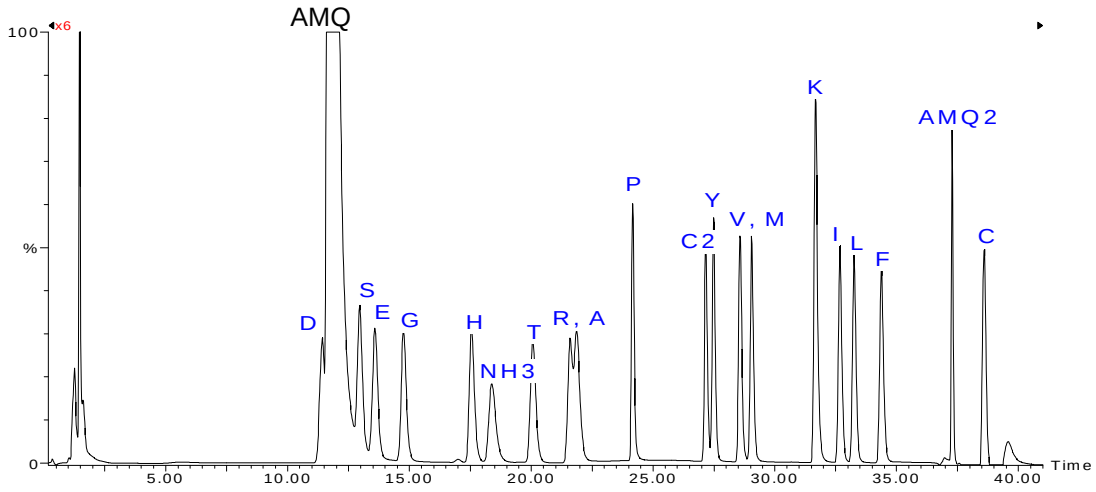
Traditional chromatographic analysis of amino acids relies solely on retention time for identification. Mass spectrometric detection can offer an additional dimension of specificity for quantitative analysis as well as provide structural information for the identification of unusual amino acids.

This note describes the results of work performed to adapt an existing fluorimetric method to mass spectrometric detection. Amino acids were converted to their 6-aminoquinolyl-N-hydroxy-succinimidyl carbamate (Waters AccQ•Tag®) derivatives and separated on a C_{18} reversed-phase column. By operating the mass spectrometer in either the full-scan or selected-ion recording (SIR) mode, qualitative and/or quantitative data may be acquired.

Analytical Conditions
The gradient separation shown in Figure 1 was done on the Waters Alliance® LC/MS System consisting of a 2690 Separations Module and 996 photodiode diode array detector and the Micromass ZMD4000 mass spectrometer.

In the full scan experiments, both positive and negative electrospray ionization (ESI) produced strong pseudomolecular ions. By alternating the polarity during acquisition, complementary data may be obtained, providing greater confidence in assignment of molecular weights. SIR (selected ion recording) were used for quantitation because of its increased sensitivity over scan mode.

Figure 1: UV Chromatogram of AccQ•Tag® Amino Acid Derivatives



D Aspartic Acid
S Serine
E Glutamic acid
G Glycine
H Histidine
T Threonine
R Arginine
A Alanine
P Proline
C2 Cystine
Y Tyrosine
V Valine
M Methionine
K Lysine
I Isoleucine
L Leucine
F Phenylalanine
AMQ 6-amino-quinoline

Column: 2.1x150mm, 35°C
Mobile Phases
A: 140 mM NH4OAc (pH 5.0)
B: Acetonitrile
C: Water

Gradient at 300 µL/min

Time	%A	%B	%C	Curve
0	100	0	0	1
0.5	99	1	0	11
18	95	5	0	6
19	91	9	0	6
29.5	83	17	0	6
33	0	60	40	11
36	100	0	0	11

Figure 2: Comparison of Positive and Negative ESI Spectra of AMQ-Proline (285 Da)

By alternating positive and negative polarity during acquisition, complementary data provides greater confidence in assignment of molecular weights.

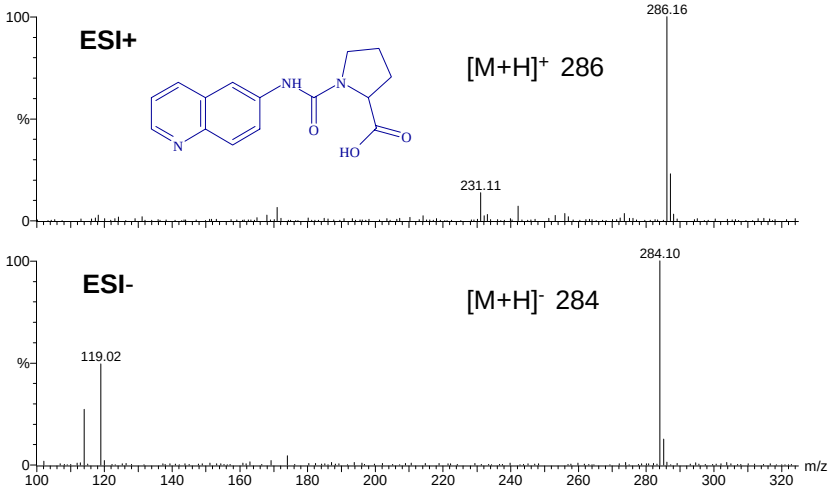


Figure 3: Comparison of Low and High Cone Voltage on the ESI+ Spectrum

The effect of alternating cone voltage on the mass spectrum of lysine (as the Di-AMQ derivative) is shown with the proposed fragment ions in the high-voltage spectrum.

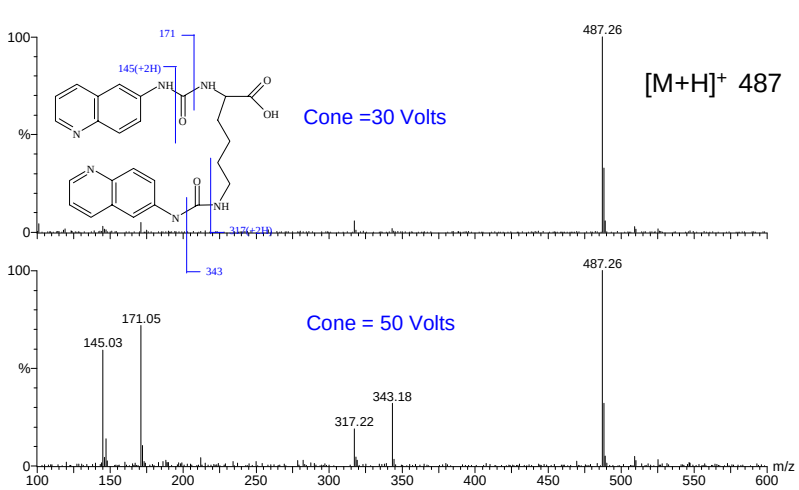
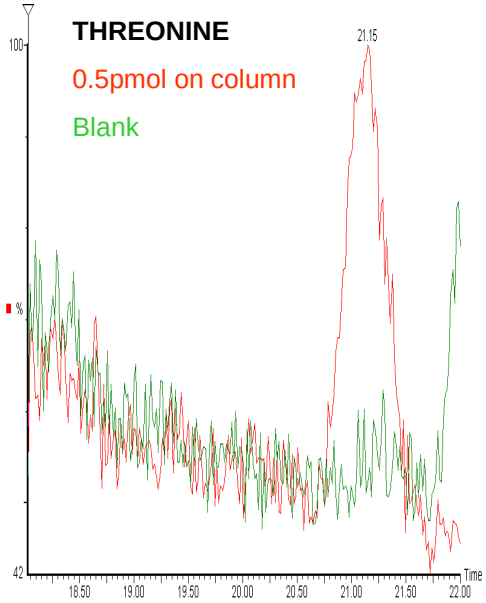


Table 1. Accuracy, Precision, and Sensitivity

Calibration curves were generated with a series of standard mixtures, (ranging from 0.1 to 50 pmol/µl) which were derivatized and analyzed under SIR conditions (500 fmol to 50 pmol on-column). Instrumental limits of detection (LOD) and quantitation (LOQ) were determined from 5 replicate injections of a 0.5 pmol/µL standard. The insert figure is an example of 0.5 pmole (500 fmol) on a Symmetry® C_{18} microbore column, 1.0x150mm at 70 µL/min flow rate.

	Mean (pmol/µL)	S.D. (pmol/µL)	% CV	%Error	LOD (pmol/µL)	LOQ (pmol/µL)
Aspartic Acid	0.381	0.155	40.8	-23.9	0.47	1.55
Serine	0.449	0.033	7.4	-10.1	0.10	0.33
Glutamic Acid	0.512	0.035	6.8	2.3	0.10	0.35
Glycine	0.479	0.106	22.1	-4.2	0.32	1.06
Histidine	0.555	0.054	9.7	11.0	0.16	0.54
Threonine	0.413	0.215	51.9	-17.3	0.64	2.15
Arginine	0.454	0.209	46.0	-9.1	0.63	2.09
Alanine	0.405	0.065	16.0	-19.1	0.19	0.65
Proline	0.460	0.025	5.5	-7.9	0.08	0.25
Cystine	0.470	0.175	37.3	-6.1	0.53	1.75
Tyrosine	0.445	0.213	47.8	-11.0	0.64	2.13
Methionine	0.362	0.256	70.6	-27.6	0.77	2.56
Lysine	0.387	0.168	43.5	-22.6	0.50	1.68
Isoleucine	0.430	0.127	29.4	-14.1	0.38	1.27
Leucine	0.000	0.000	223.6	-100.0	0.00	0.00
Phenylalanine	0.470	0.017	3.6	-6.1	0.05	0.17.



Conclusions: This study illustrates the flexibility and sensitivity that mass spectrometry offers in the analysis of amino acids. It demonstrates that 6-aminoquinolyl-N-hydroxy-succinimidyl carbamate derivatives are extremely well-suited to LC/MS analysis. In addition to their stability and robust chromatographic properties, they yield very strong mass spectra under both the positive and negative electrospray ionization. Instrumental sensitivities of the ZMD, on the order of a few picomoles on-column, are demonstrated. In addition to these quantitative results, the use of mass spectrometric detection can provide the analytical chemist with the means to perform experiments unavailable with traditional UV or fluorescence detectors, such as the identification of unusual amino acids through the use of “in-source CID,” and the determination of stable-isotope incorporation in metabolic studies.

