Application Note: 370

Separation and Quantitation of Complex Amino Acid Derivatives in Plasma

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Key Words

- MSQ Plus™
- Hypersil GOLD™ Columns
- Amino Acid Analysis
- LC/MS
- Single Quadrupole MS

Goal: To develop a direct LC/MS screening method to separate, identify, and measure multiple amino acid derivatives (AADs) in plasma by single quadrupole mass spectrometry.

Introduction

Amino acids and their derivatives are important biomarkers in human physiological processes. Their identification and quantitation can provide significant insights related with human health. In this application, we report the simultaneous analysis of 40 amino acid derivatives in plasma by single quadrupole LC/MS. The Thermo Scientific MSQ Plus was selected for this application to take advantage of its rapid scanning abilities of up to 12,000 amu/sec and multiple grouping capabilities for fast data acquisition necessary for multiple Single Ion Monitoring (SIM) experiments.

Methods

Instruments

The method was developed using a Thermo Scientific Surveyor Plus LC/MS platform comprising of a Thermo Scientific Surveyor LC Pump Plus; Thermo Scientific Surveyor Autosampler Plus; and an MSQ Plus single quadrupole mass spectrometer with electrospray ionization (ESI). Direct infusion was used for instrument tuning to optimize the mass spectrometer's settings. Analytical separations were achieved using a 50 × 2.1 mm reversed-phase Thermo Scientific Hypersil GOLD column with 3 micron particles. In this analysis, multiple sets of samples containing mixtures between 6 to 40 AADs in plasma were utilized.

HPLC Conditions

Binary gradient profiles were developed using water with 25 mM HCOOH/NH_4OH (A) and 60% acetonitrile/40% water (B) at a flow rate of 300 µL/min with a 10 mL partial loop injection. A primary group of 40 amino acid derivatives was separated and detected in 20 minutes with a gradient spanning 5% to 95% (B). A subset of six amino acid derivatives was selected for rapid quantitative, linearity, and reproducibility studies using a 10 minute gradient spanning 5% to 95% (B). Sample tray temperature was set at 25 °C and column oven temperature was set at 40 °C.

MSQ Plus Setup

Positive polarity electrospray ionization was used for the analysis of the amino acid derivatives. MS method development was performed using direct infusion into the mass spectrometer, while all analyses included chromatographic separation prior to the MS detection. A full scan and many SIM scans were used for MS parameter optimization, however analytical work was done using multiple SIM scans collected in a single group (Figure 1).

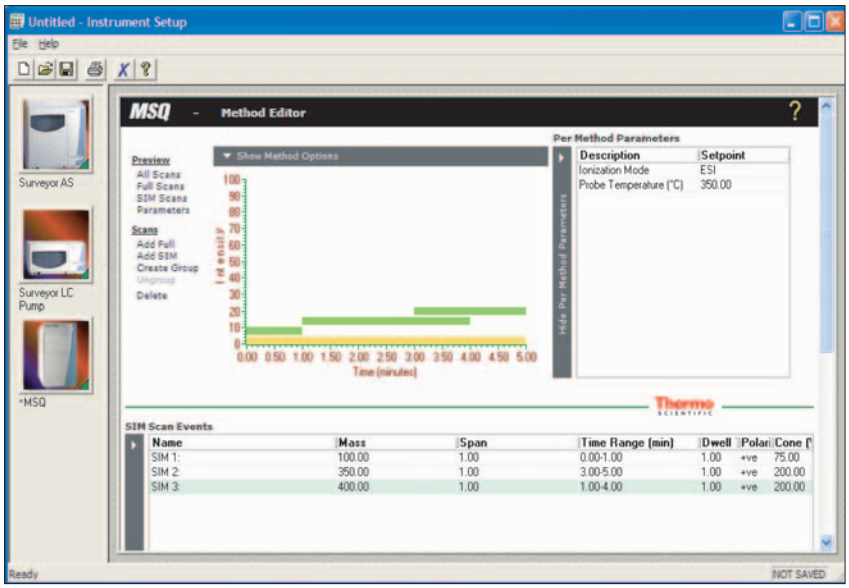

Figure 1: MSQ Plus method editor with graphical representation of one full scan (yellow bar) and three SIM scans (green bars) with overlapping time segments

Simultaneous Monitoring of 40 AADs

Up to 40 SIM scans were grouped for high speed detection of all AADs in the sample analyzed (Figure 2). In order to maximize data acquisition, the settings for the analyzer quad for a given mass were held to a narrow window width of 0.2 amu for about 20 ~100 millisecond (ms), then the settings were changed to another mass, and so on for all 40 different SIM settings. A single group of multiple SIM scans was created in lieu of multiple individual SIMs in order to provide a single Total Ion Chromatogram (TIC) data file. The 40 individual data files of ungrouped SIMs would significantly slow the data acquisition speed, thereby compromising data points for rigorous quantitative analysis. This technique provides ample data for post-acquisition processing while maintaining maximum acquisition speed.

Results

Analysis of AADs

The TIC from the combined 40 SIM channels is presented in Figure 3A. Even though only one TIC was acquired, all of the individual AADs can be easily monitored by extracted Selected Ion Chromatograms (SIC) from the TIC using post acquisition data processing. For example, Figure 3B shows the signal intensity of a derivative of Lysine. Although this peak is barely visible in the TIC in Figure 3A, the selectivity of the extracted trace provides a quantitative peak area of 424394 with a signal to noise ratio (S/N) of 254. Comparison of this SIC to the earlier eluting peaks in the TIC demonstrates the wide dynamic range achieved by collecting and grouping multiple SIMs.

This method is suitable for trace level components, as well as chromatographically complex mixtures. The first three minutes of the TIC, shown in Figure 4A, contain multiple unresolved peaks. Extracted examples from that group are five representative SIC peaks of the derivatives of Glutamine/Glutamic Acid (4B), Alanine and Alanine-d3 (4C), and Proline (4D). The complete analysis can be similarly performed for all 40 different AADs for quantitative analysis.

Reproducibility and Linearity Measurements

The reproducibility measurements were done using six AAD samples with a total sample concentration of 50 µM. The results showed standard deviation values <3% for quantitation analysis illustrated in Figure 5.

Linearity measurements over four orders of magnitudes were determined over a total concentration range from 0.1 µM to 1000 µM (0.1, 0.5, 1, 5, 10, 50, 100, 500, 1000) with R^2 = 0.9991 to establish the working range of the method shown in Figure 6A. A second calibration curve with emphasis on the lower concentration range from 0.1 µM to 10 µM (0.1, 0.25, 0.5, 1, 5, 10) with R^2 = 0.9986 was developed to reduce the high-concentration bias in order to verify linearity in the expected working range illustrated in Figure 6B. LC/MS results are the average of five replicates for each concentration. This excellent linearity over a low concentration range is particularly useful for biomarker detection and quantitation.

Time (minutes)

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SIM Scan Events

Show Full Scans

Name	Mass	Span	Time Range (min)	Dwell Tim	Polarity	Cone (V)
GROUP 1:			0.00-30.00		+ve	70.00
SIM 1:	137.90	0.20	0.00-30.00	0.10	+ve	70.00
SIM 2: 182.3	182.20	0.20	0.00-30.00	0.10	+ve	70.00
SIM 3:	195.90	0.20	0.00-30.00	0.10	+ve	70.00
SIM 4:	197.90	0.20	0.00-30.00	0.10	+ve	70.00
SIM 5:	210.16	0.20	0.00-30.00	0.10	+ve	70.00
SIM 6:	212.80	0.20	0.00-30.00	0.10	+ve	70.00
SIM 7:	224.07	0.20	0.00-30.00	0.10	+ve	70.00
SIM 8:	226.00	0.20	0.00-30.00	0.10	+ve	70.00
SIM 9:	236.04	0.20	0.00-30.00	0.10	+ve	70.00
SIM 10:	238.04	0.20	0.00-30.00	0.10	+ve	70.00
SIM 11:	239.90	0.20	0.00-30.00	0.10	+ve	70.00
SIM 12:	245.95	0.20	0.00-30.00	0.10	+ve	70.00
SIM 13:	252.01	0.00	0.00-30.00	0.10	+ve	70.00
SIM 14:	253.01	0.20	0.00-30.00	0.10	+ve	70.00
SIM 15:	254.00	0.20	0.00-30.00	0.10	+ve	70.00
SIM 16:	256.90	0.20	0.00-30.00	0.10	+ve	70.00
SIM 17:	266.95	0.20	0.00-30.00	0.10	+ve	70.00
SIM 18:	268.04	0.20	0.00-30.00	0.10	+ve	70.00
SIM 19:	270.13	0.20	0.00-30.00	0.10	+ve	70.00
SIM 20:	274.00	0.20	0.00-30.00	0.10	+ve	70.00
SIM 21:	274.00	0.20	0.00-30.00	0.10	+ve	70.00
SIM 22:	276.10	0.20	0.00-30.00	0.10	+ve	70.00
SIM 23:	282.00	0.20	0.00-30.00	0.10	+ve	70.00
SIM 24:	286.07	0.20	0.00-30.00	0.10	+ve	70.00
SIM 25:	290.10	0.20	0.00-30.00	0.10	+ve	70.00
SIM 26:	291.00	0.20	0.00-30.00	0.10	+ve	70.00
SIM 27:	295.14	0.20	0.00-30.00	0.10	+ve	70.00
SIM 28:	295.90	0.20	0.00-30.00	0.10	+ve	70.00
SIM 29:	299.00	0.20	0.00-30.00	0.10	+ve	70.00
SIM 30:	302.07	0.20	0.00-30.00	0.10	+ve	70.00
SIM 31:	325.11	0.20	0.00-30.00	0.10	+ve	70.00
SIM 32:	347.11	0.20	0.00-30.00	0.10	+ve	70.00
SIM 33:	361.10	0.20	0.00-30.00	0.10	+ve	70.00
SIM 34:	373.10	0.20	0.00-30.00	0.10	+ve	70.00
SIM 35:	373.17	0.20	0.00-30.00	0.10	+ve	70.00
SIM 36:	387.10	0.20	0.00-30.00	0.10	+ve	70.00

Figure 2: 40 grouped SIM scan events optimized for rapid AAD analysis. Each SIM was assigned to monitor one AAD

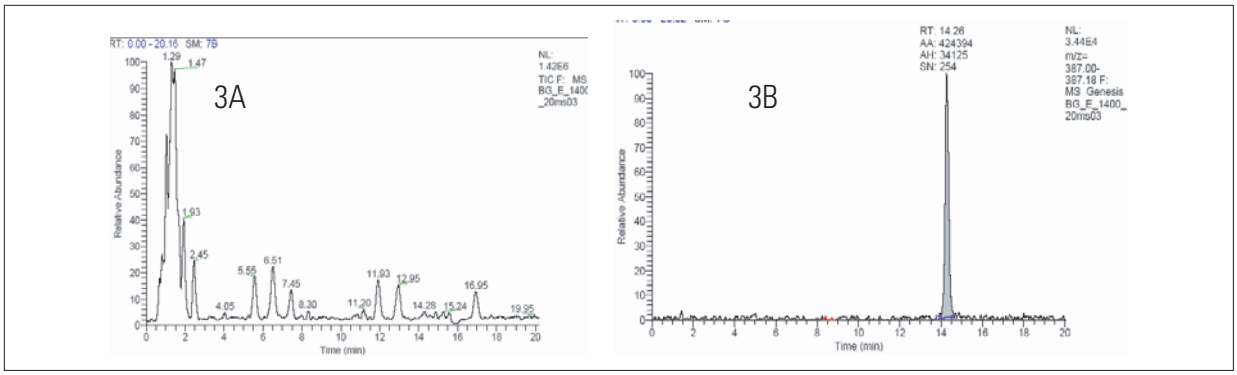

Figure 3: TIC from 40 grouped SIM scans for the samples containing 40 AADs in plasma (3A), with a total sample concentration of 50 µM. The signal to noise ratios (S/N) of the AADs are ranging from 63.7 to 3,211 (RMS), well above the threshold for quantitative analysis. Figure 3B shows the SIC for lysine derivative extracted from 3A, where peak area and S/N are suitable for quantitative analysis, even though it appears as a trace level component in the TIC in Figure 3A

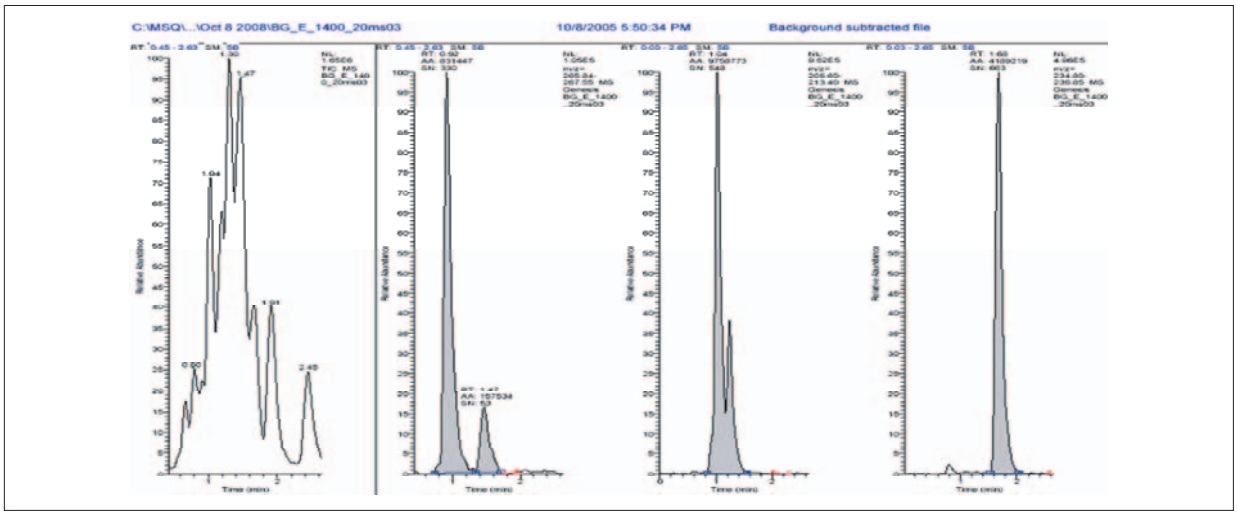

Figure 4: The early elusion (time 0-2 min) of 40 AAD analysis from Figure 3A is shown in 4A.
The extracted SICs of the derivatives of Glutamine/Glutamic Acid (4B), Alanine and Alanine-d3 (4C), and Proline (4D)

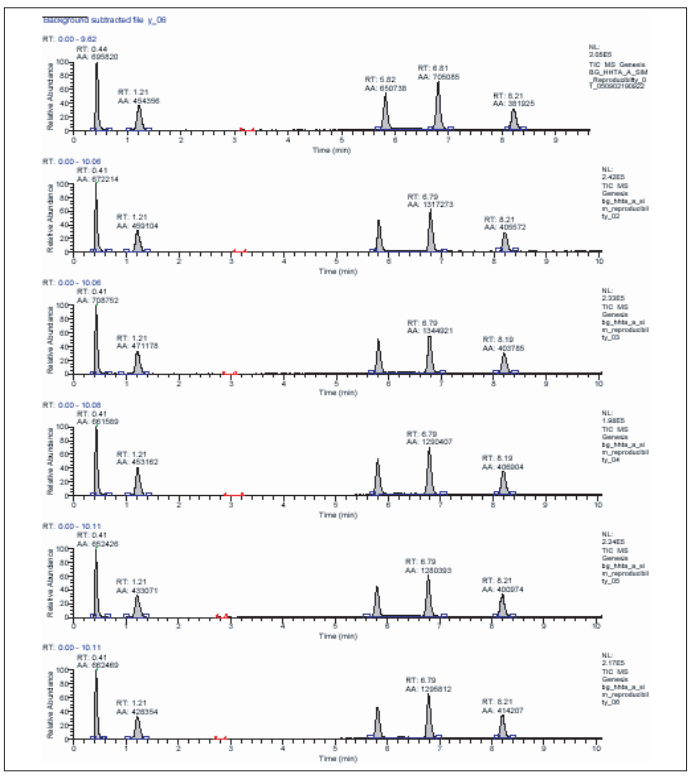

Figure 5: Reproducibility of six different HPLC runs of the six amino acid derivative mixture

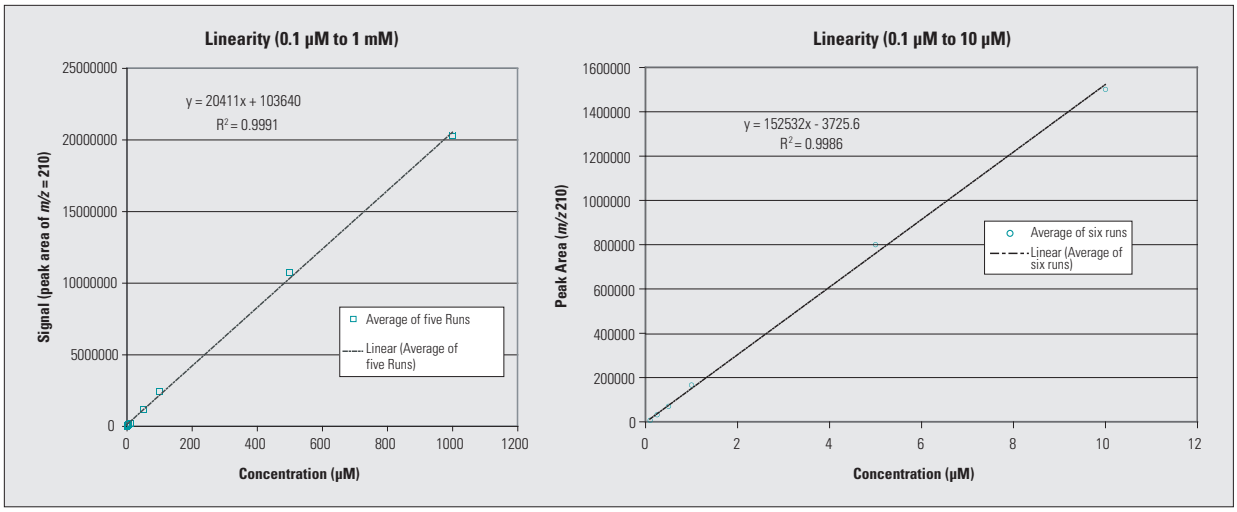

Figure 6: Linearity measurements of a 6 amino acid derivative mixture over a dynamic range of 4 orders of magnitudes (A). The calibration curve with emphasis on the low concentration range from 0.1 µM to 10 µM (B)

Conclusions

A fast screening method was established for Amino Acid Derivatives using a Surveyor Plus HPLC and MSQ Plus single quadrupole mass spectrometer. This method was used to simultaneously measure 40 AADs in plasma resulting in quantitative data, significant sensitivity enhancement, and a wide linear dynamic range spanning four orders of magnitude.

The rapid analysis of all 40 AADs was achieved using the fast scan speeds of the MSQ Plus combined with the capability of grouping the individual SIM scans for each compound to produce a single TIC. Quantitative analysis was performed by a post-acquisition processing of the TIC, extracting the original SICs of the individual amino acid derivatives to maximize data acquisition speed.

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AN62545_E 12/07S