Application Note

LC/MS DETERMINATION OF AMINO ACIDS

Using Compact Mass Spectrometry

Advion

INTRODUCTION

Amino acids are the building blocks of the protein backbone and universally contain an amine and carboxylic acid functional group. Free amino acid blood determination is important for the early detection of inborn errors of metabolism in the newborn screening program. Also, amino acid usage has increased in the food industry and requires product control analysis.

HPLC is the most common separation technique for the analysis of samples containing amino acids, with traditional approaches using pre or post column derivatization and UV detection. However, derivatization is adding unnecessary sample preparation steps and using mass spectrometry as the detector allows for overlapping analyte peaks and hence faster HPLC analysis times.

Here, we show examples for the determination of amino acids on a combined HPLC compact mass spectrometry (LC/CMS) system for the rapid detection and quantification of amino acids utilizing a novel mixed mode LC column. This sample analysis strategy results in a cost effective analysis system to detect and quantify free amino acids in mixtures, protein amino acid compositions and amino acid biomarkers.

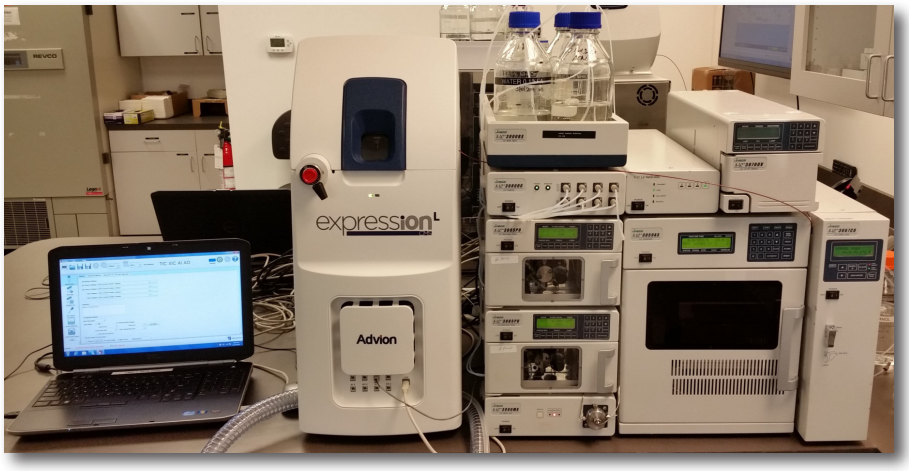

Figure 1: UHPLC-CMS set up for the detection and quantification of amino acids in complex mixtures utilizing a JASCO X-LC system (Jasco, USA) , an Intrada Amino Acid HPLC column (Imtakt Corporation, Japan) and the expression^{L} compact mass spectrometer (Advion, Inc. USA).

METHOD

LC method using a 150 mm x 2mm Intrada column with 3 um particles (Imtakt Corporation, Japan) and a flow rate of 200-400 µL/min. Solvent A: Acetonitrile/Tetrahydrofuran/25 mM Ammonium formate/Formic Acid 9/75/16/0.3 (v:v:v:v) and Solvent B: Acetonitrile/100 mM Ammonium formate 20/80 (v:v).

RESULTS

A first separation example is shown in Figure 1 with a 60 min chromatography separation and LC-CMS detection of a standard 20 amino acid mixture using selected ion monitoring (SIM) acquisition for each analyte. Here, a complete baseline separation can be achieved and isotopic analytes such as Leucine and Iso-Leucine are baseline separated and readily distinguishable by their unique retention times.

However, due to the selectivity of the CMS detector, a baseline separation of all analytes is not necessarily required and faster chromatography can be used to analyze the 20 amino acid mixture in only 15 min (Figure 2) – a significant increase in sampling speed. Now, the Leu/Ile pair is still resolved, albeit not baseline separated, and some other analytes are co-eluting. But since the co-eluting peaks have different mass-to-charge ratios they can easily be distinguished by mass spectrometry, something not possible in UV detection with or without derivatization.

Figure 3 shows example calibration functions for four amino acids (Proline, Tyrosine, Phenylalanine and Threonine) with good linearity in the range of 5 to 1000 pmol on-column (ca. 600 pg to 180 ng of the respective amino acid). This analysis employed the 15 min chromatography for a faster sample throughput. Observed linearity and sensitivity is well within the required levels for eg. amino acid blood determination.

To further show the potential of the LC-CMS analysis system, Figure 4 shows the separation and detection gamma-aminobutyric acid (GABA) isomers. All three isotopic and isobaric analytes are baseline-separated and can be readily distinguished. The same can be shown for a mixture of isotopes of Leucine (Figure 5). Here, the mobile phase solvents had to be slightly adjusted and gradient conditions changed as indicated to achieve a separation of all three isotopes.

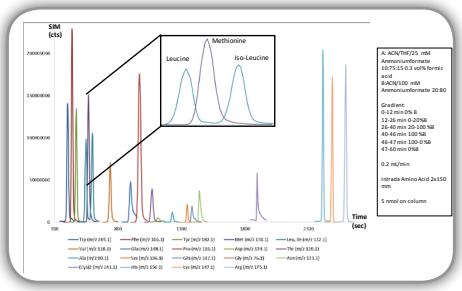

Figure 2: Example of a 60 min LC-CMS analysis of a 20 amino acid mixture using SIM (5 nmol on column).

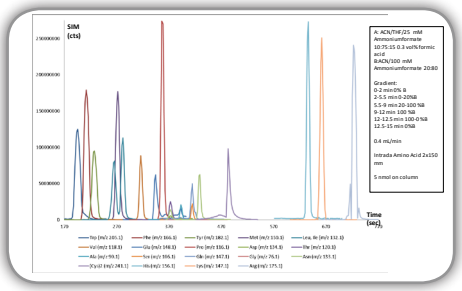

Figure 3: Example of a 15 min LC-CMS analysis of a 20 amino acid mixture using SIM acquisition of the unique m/z of each amino acid.

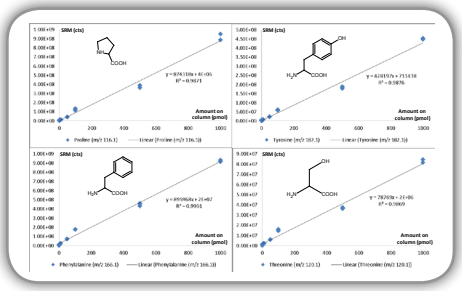

Figure 4: Example calibration functions for four amino acids: Proline, Tyrosine, Phenylalanine and Threonine (15 min LC-CMS method).

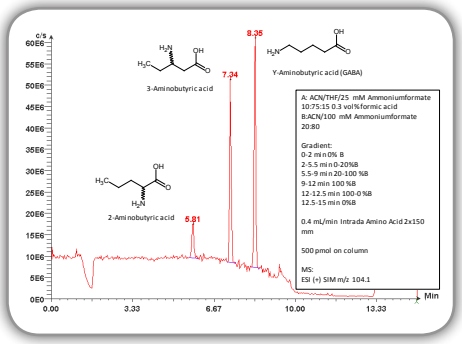

Figure 5: Separation of GABA isomers using the same 15 min LC-CMS method than before

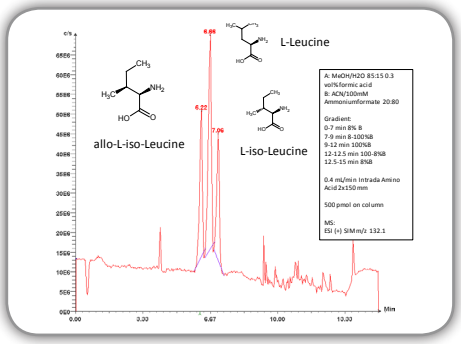

Figure 6: Separation of L-Leucine isomers using a modified15 min gradient

CONCLUSIONS

A combination of ion-ion interaction chromatography and a compact mass spectrometer presents a cost effective and powerful analysis system for the detection and quantification of amino acids in complex mixtures without the need for extra sample processing steps such as derivatizations.

ACKNOWLEDGEMENT

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