Rapid LC-MS/MS analysis of free amino acids in extracellular matrix

Quantitative, fast, sensitive and robust analysis on the QTRAP® 6500+ System

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A rapid, robust and simple method is described for the quantitative measurement of 17 amino acids without prior derivatization. The method was first developed on standard samples as shown in Figure 1, showing the MRM extracted ion chromatograms for 17 amino acids. The method was applied to the analysis of cell supernatant from purified peripheral blood mononuclear cells (PBMCs) (Figure 2, top).

Using the unique functionality of the QTRAP System, MRM triggered MS/MS known as the MIDAS™ Workflow, analytes can be detected, identified and quantified in a single run. In this workflow, a set of MRM transitions are used as a survey scan to trigger the acquisition of high sensitivity linear ion trap MS/MS data. These data allow conclusive identification of analytes, including isobaric structures.

MRM extracted ion chromatograms for four amino acids at concentrations close to their limits of detection (LODs), and corresponding linearity data, are shown in Figures 3 and 4, respectively. LODs were calculated for 17 amino acids, and their concentrations measured in diluted PBMC cell supernatant (Table 2).

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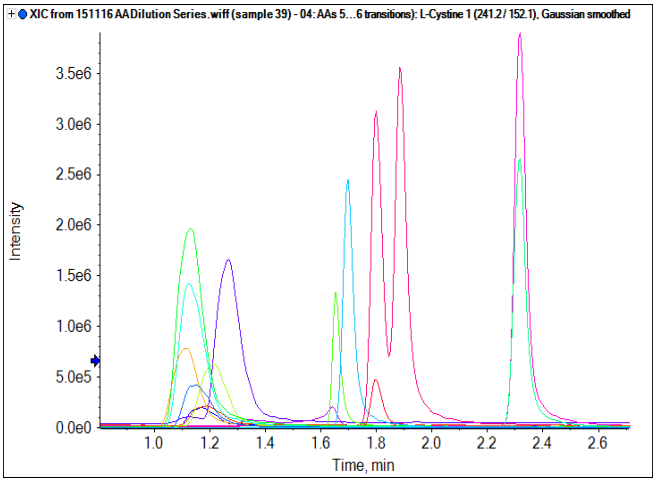

Figure 1. Targeted MRM analysis of amino acids. MRM extracted ion chromatograms (XICs) for 17 amino acids, each at 0.5 pmol on-column (except for cystine, 0.25 pmol on-column). Method was developed on underivatized standard samples.

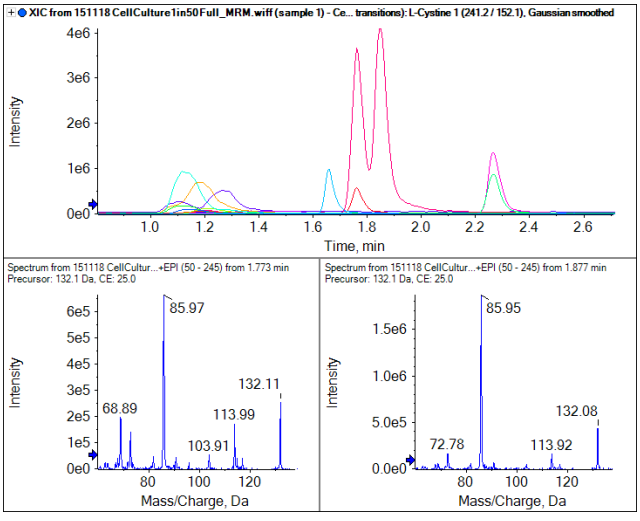

Figure 2. The MIDAS™ Workflow. Top panel, MRM extracted ion chromatogram for 17 amino acids in PBMC cell supernatant (diluted 1 in 50). Bottom panel, left to right, full scan linear ion trap MS/MS spectrum generated at 1.75 min and 1.88 min, corresponding to isobaric amino acids L-isoleucine and L-leucine. The L-isoleucine diagnostic fragment ion at m/z 69 can be observed in the MS/MS spectrum on the left.

Methods

Sample preparation: Amino Acid standard solution (AAS18, Sigma), containing 2.5 µmoL/mL each of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine·HCl, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine, and 1.25 µmoL/mL L-cystine. Sample was diluted for analysis in 0.1% formic acid in water. Cell supernatant from purified PBMCs (10,000,000 cells) was subjected to methanol precipitation, and the supernatant removed. Supernatant was diluted 1 in 50 in 0.1% formic acid in water for analysis.

Chromatography: MicroLC 200 System (Eksigent), with ACE 3 AQ column (HiChrom), 0.5 x 150 mm; flow rate 20 µL/min. Mobile phases: 0.1% formic acid in water (A), 0.1% formic acid in acetonitrile (B). Gradient: 2 to 20% B over 5 minutes, total analysis time 10 minutes. Injection volume 2 µL.

Mass spectrometry: QTRAP® 6500+ LC-MS/MS System (SCIEX), with IonDrive™ Turbo V Source equipped with 50 µm i.d. electrode. Data acquired in MRM (Table 1) or MIDAS™ Workflow mode. Peak integration using MultiQuant™ Software v3.0.2.

Table 1. Optimized MRM transitions for 17 free amino acids.

Amino Acid	Q1 (m/z)	Q3 (m/z)	DP (V)	EP (V)	CE (V)	CXP (V)
Glycine	*76.1*	*30*	*6*	*7.6*	*19*	*14*
L-Alanine	*90.1*	*44*	*6*	*4.5*	*17*	*6*
L-Serine	*106.1*	*60*	*6*	*10.5*	*15.5*	*7*
L-Proline	*116.1*	*70*	*20*	*13.5*	*21*	*10*
L-Valine	*118.1*	*55*	*11*	*13.5*	*27*	*8*
L-Threonine	*120.1*	*103.2*	*105*	*14.5*	*25*	*7*
L-Leucine/isoleucine	*132.1*	*86*	*8*	*14.5*	*13*	*10*
Isoleucine 2	*132.1*	*69*	*8*	*14.5*	*23*	*8*
L-Aspartic Acid	*134.1*	*74*	*7*	*14.5*	*19*	*10*
L-Lysine	*147.1*	*84*	*15*	*13.5*	*23*	*10*
L-Glutamic Acid	*148.1*	*84*	*21*	*14.5*	*21*	*10*
L-Methionine	*150.2*	*104*	*6*	*12*	*15*	*12*
L-Histidine	*156.1*	*110*	*16*	*13*	*19*	*12*
L-Phenylalanine	*166.1*	*103*	*11*	*14*	*37*	*12*
L-Arginine	*175.2*	*70*	*40*	*11*	*27*	*8*
L-Tyrosine	*182.1*	*165.2*	*20*	*11*	*13*	*8*
L-Cystine	*241.2*	*152.1*	*20*	*14*	*19*	*10*

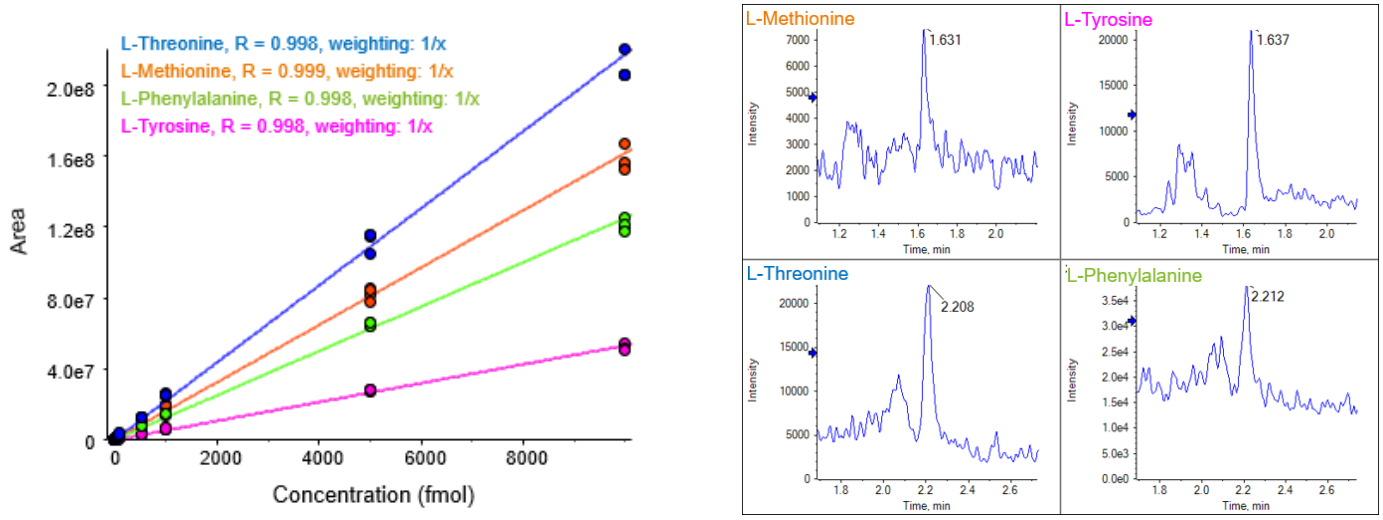

Figure 3. Quantitation curves for selected amino acids. (Left) Linearity for L-threonine, L-methionine, L-phenylalanine and L-tyrosine over 4 orders of magnitude linear dynamic range (1 fmol on-column to 10 pmol on-column). (Right) Signal observed for these same amino acids, each at 1 fmol on column.

Conclusions

The method described here allows rapid, robust and sensitive quantitation of 17 amino acids without derivatization. Use of the MIDAS workflow, unique to QTRAP Systems, allows unambiguous analyte identification. The method has been successfully applied to the quantitation of amino acids in a sample of PBMC cell supernatant.

Table 2. Limits of detection for 17 free amino acids and their concentrations measured in diluted PBMC cell supernatant (1 in 50).

Amino Acid	LOD (fmol)	Mean conc (n=3) diluted PBMC (fmol/µL)	Peak Area %CV	Calc conc undiluted PBMC (pmol/µL)
Glycine	*<1000*	*<LOD*	-	-
L-Alanine	*<1000*	*<LOD*	-	-
L-Serine	*50*	*21*	*7.7*	*1.0*
L-Proline	*2.5*	*96*	*4.7*	*4.8*
L-Valine	*25*	*105*	*6.1*	*5.2*
L-Threonine	*1*	*97*	*7.3*	*4.9*
L-Leucine	*2.5*	*338*	*3.2*	*17*
Isoleucine	*2.5*	*329*	*1.5*	*16*
L-Aspartic Acid	*10*	*26*	*4.9*	*1.3*
L-Lysine	*5*	*305*	*2.2*	*15*
L-Glutamic Acid	*5*	*55*	*4.8*	*2.8*
L-Methionine	*1*	*3*	*6.2*	*0.17*
L-Histidine	*5*	*23*	*4.0*	*1.1*
L-Phenylalanine	*1*	*100*	*5.1*	*5.0*
L-Arginine	*2.5*	*220*	*5.4*	*11*
L-Tyrosine	*1*	*97*	*1.4*	*4.8*
L-Cystine	*1.25*	*36*	*6.8*	*1.8*

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