Free Amino Acids in Extracellular Matrix

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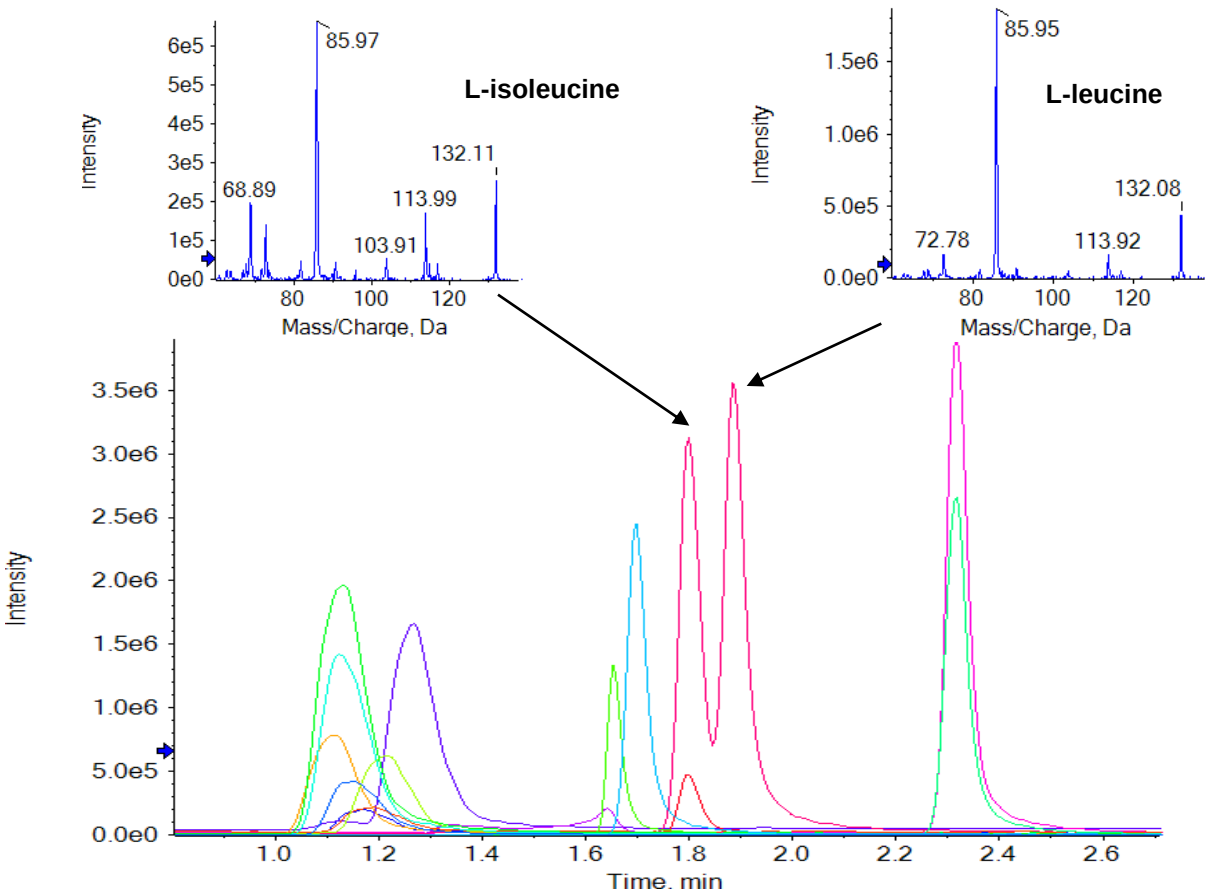
Conditions

Column:	ACE 3 AQ
Dimensions:	150 x 0.5 mm
Part Number:	ACE-116-15005
Mobile Phase:	A: 0.1% (v/v) formic acid in H_2O B: 0.1% (v/v) formic acid in MeCN

Time (mins)	%B
0	2
5	20

Flow Rate:	20 µL/min
Injection:	2 µL
Detection:	SCIEX QTRAP 6500 LC-MS/MS system IonDrive Turbo V source Positive ion MRM mode
Sample:	Standard solution containing 2.5 µmol/mL each amino acid (1.25 µmol/ mL cysteine). 0.5 pmol on-column (except for cysteine, 0.25 pmol on-column). Method also applied to analysis of cell supernatant from purified peripheral blood mononuclear cells (PBMCs)

Full scan linear ion trap MS/MS data can distinguish isobaric amino acids L-isoleucine and L-leucine.



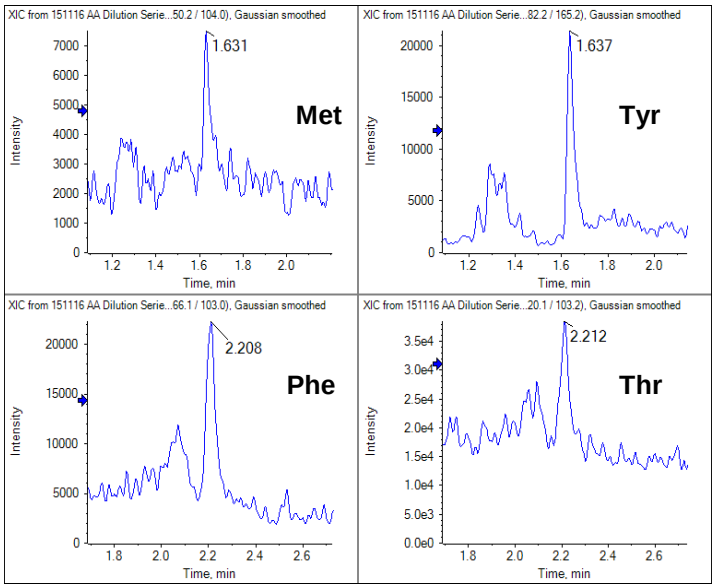
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MRM transitions and limits of detection (LODs) for 17 free amino acids and their concentrations measured in diluted PBMC cell supernatant

Peak	Analyte	Rt (mins)	MRM Transition (*m/z*)	LOD (fmol)	PBMC cell conc. (fmol/µL)
1	Lys	1.094	147.1 → 84	5	305
2	His	1.111	156.1 →110	5	23
3	Arg	1.117	175.2 →70	2.5	220
4	Gly	1.129	76.1 → 30	<1000	<LOD
5	Cys	1.140	241.2 → 152.1	1.25	36
6	Asp	1.155	134.1 → 74	10	26
7	Ser	1.156	106.1 → 60	50	21
8	Ala	1.189	90.1 → 44	<1000	<LOD
9	Glu	1.208	148.1 →84	5	55
10	Pro	1.262	116.1 → 70	2.5	96
11	Val	1.630	118.1 → 55	25	105
12	Met	1.645	150.2 →104	1	3
13	Tyr	1.669	182.2 →165.2	1	97
14	Ile	1.773	132.1 → 86, 69	2.5	329
15	Leu	1.858	132.1 → 86	2.5	338
16	Phe	2.273	166.1 → 103	1	100
17	Thr	2.275	120.1 → 103.2	1	97

MRM extracted ion chromatograms for four amino acids each at 1 fmol on-column



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