Current Standard Operating Protocols (SOP), NCBS-CCAMP MS-Facility

Metabolomics –Quantification of Amino acids

Purpose: To provide general guidelines for conducting the quantification of amino acids using tandem triple quadrupole mass spectrometer.

Reagents: All solvents and reagents used are of LC-MS quality.

Protocol:

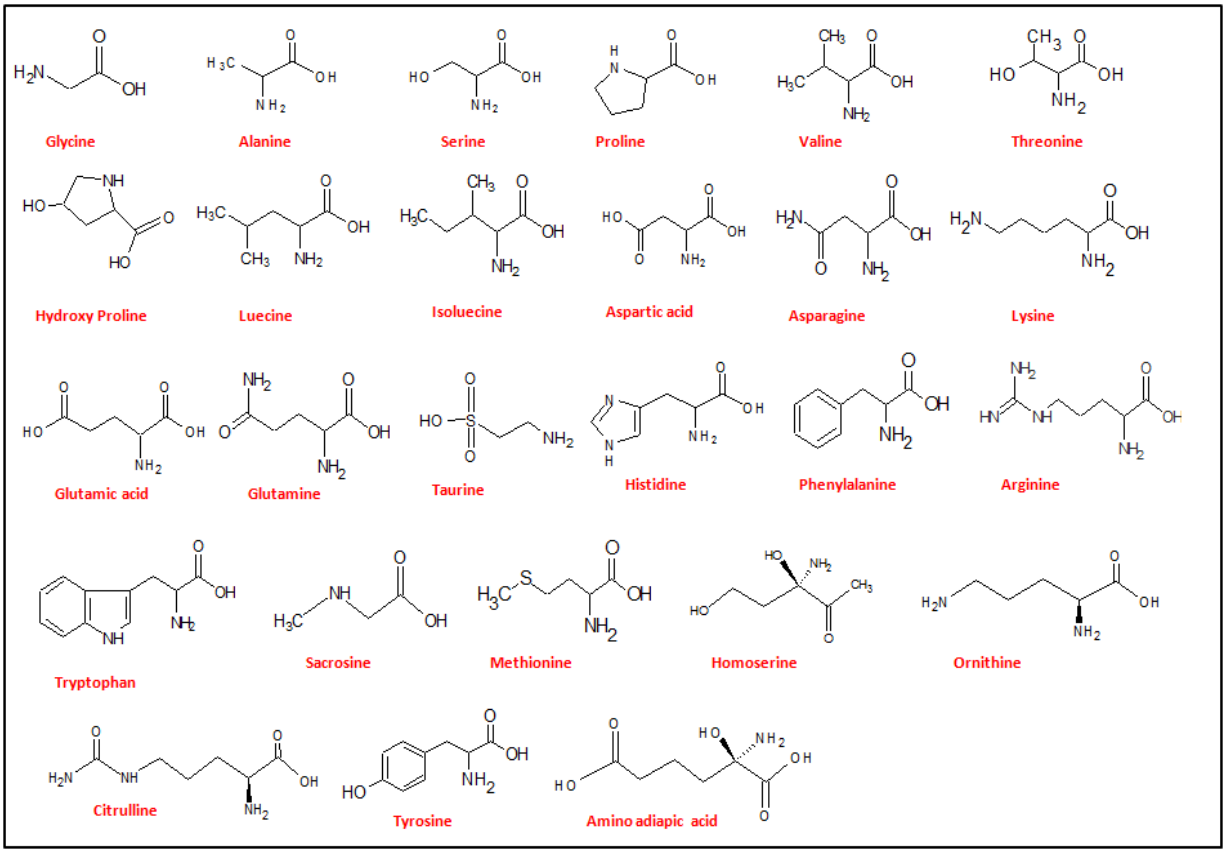

Figure 1: List of all twenty six amino acids.

A. Sample Preparation:

- Prepare the individual stock solutions of standards and internal standards each (~1mg/mL) in 0.1 N HCl.
- Prepare 10µg/mL stocks of both STDs and ISTDs in 0.1N HCl by taking the required amount (10 µL) from the individual stock.

- Prepare 5µg/mL stocks of both STDs and ISTDs in 0.1N HCl by diluting further from the 10µg/mL stock.
- Derivatizing reagent AQC was synthesised using the raw materials N,N′-Disuccinimidyl carbonate (DSC) and 6-Aminoquinoline (AMQ) in equimolar concentration.
- Prepare 1mg/mL of AQC in 100% ACN in a vial. Heat the vial on top of a heating block (55˚C), vortex occasionally until it dissolves (Do not heat longer than 10min).
- Dissolved AQC stock has to be stored in a desiccator and it is stable approximately for a month (cannot be used if it is turned to yellow brown colour).
- Prepare 1.76mg/ml of Ascorbic acid in water and vortex until it dissolves (This is a reducing agent, it is added during the reaction to keep methionine in a stable condition).
- Derivatization
    - Preheat the vortex mixer (incubator shaker) to 55˚C.
    - 70µL of borate buffer of pH 8.8 is taken to which 10µL of STDs, ISTDs, ascorbic acid and 10µg of the derivatizing reagent AQC is added.
    - This mixture is kept on the mixer at 350 rpm for exact 10min.
    - The reaction is stopped after 10 min by the addition of formic acid to reduce the pH as the above reaction is pH and temperature specific.
- After derivatization load onto the RP-SPE (30mg/1ml, Strata cartridges) columns with 500µL of H_2O followed by subsequent wash steps and final elution with The column procedure is as follows:
    - Activate the column with 1mL methanol
    - Equilibrate with 1mL water(0.1% Formic acid)
    - Load the sample
    - Wash with water (0.1% Formic acid), repeat 2-3 times
    - Elute with 1mL ACN:MeOH in the ratio 20:80 and 1% formic acid.
- Dry (Speed Vac for 2-3 hours) and reconstitute with 50µL of starting gradient i.e. 2% ACN.
- Vortex and centrifuge the reconstituted sample.

- Further transfer the supernatant into the HPLC vial and place it in the auto sampler for the analysis.

B. LC-SRM Analysis:

- Equilibrate the C-18 column (2.1 × 100 mm, 1.8 µm, Phenomenex, Inc) with 2% acetonitrile.
- Use the mobile phase solvents A: water (10 mM ammonium acetate, 0.1 % FA), B: Acetonitrile (0.1% FA) with the flow rate of 200 µL/min for the analysis.
- Set the following gradient (2% B at 0 min, 2% B at 3 min, 20% B at 20min, 35% B at 25 min, 80% B at 25-27 min, 2% B at 27-30 min) in the LC system.
- Set operating conditions as follows: spray voltage-3700V; ion transfer capillary temperature-270°C; source temperature- 30 °C; sheath gas-1, auxillary gas-10 (arbitrary units); collision gas-argon; S-lens voltage and collision energy as per table 1; scan time of 50 millisec/transition; and ion polarity positive.
- Select the most intense product ion corresponding collision energy and S-lens voltage of each for the LC-SRM analysis as shown in the table 1.
- Inject 10µL (10 ng on column) into LC-MS for analysis.
- The expected result is shown in the figure 2.

	Name	Parent ion (m/z)	Product ion (m/z)	Collision Energy (CV)	S-lens Voltage	Retention time (min)
1	4-Hydroxy proline	302.1	171	21	85	6.15
	4-Hydroxy proline D3	305.32	171	22	85	6.06
2	Histidine	326.11	110.1	25	82	7.26
	Histidine D3	329	113.1	26	82	7.15
3	Asparagine	303.1	171	20	81	7.45
4	Taurine	296.3	171.1	20	65	8.13
	Taurine D4	300.34	171.1	25	70	8.1
5	Serine	276.1	171.04	21	84	8.19
	Serine D3	279.2	171.1	20	92	8.16
6	Glutamine	317.8	171	25	85	8.45
	Glutamine D5	322.2	171.1	28	94	8.4
7	Arginine	345.1	171	29	97	8.83
8	Homoserine	290.05	171.01	20	82	8.95
9	Glycine	246.03	171.06	22	82	9.05
	Glycine D2	248.1	171.07	18	83	9.02
10	Aspartic acid	304.1	171.04	21	98	9.31
	Aspartic acid D3	307.1	171.02	21	96	9.28
11	Sacrosine hydrochloride	260.1	171	24	78	9.92
	Sacrosine hydrochloride D5	265.26	171	19	74	9.83
12	Citrulline	346.1	171	27	84	10.18
	Citrulline D7	353.4	171	23	86	10.13
13	Glutamic acid	317.8	171	25	85	10.25
	Glutamic acid D5	323.26	171	21	85	10.19
14	Threonine	290.05	171.01	20	82	10.8
	Threonine D2	292.3	171.02	25	70	10.76
15	Alanine	260.1	171	24	78	11.91
16	Amino adiapic acid	332.13	171	29	89	12.72
	Amino adiapic acid D3	335.35	171	29	89	12.65
17	Proline	286.07	171.04	23	75	13.21
	Proline D3	289.32	171.04	23	75	13.18
18	Ornithine	237.1	171.1	15	67	15.62
	Ornithine D7	240.7	171.1	20	68	15.53
19	Lysine	244.2	171.1	15	72	16.95
	Lysine D8	248.3	171.1	17	62	16.85
20	Tyrosine	352.2	171	22	117	17.01
	Tyrosine D4	359.4	171	22	117	16.89
21	Methionine	320.06	171.03	23	104	17.6
22	Valine	288.2	171	21	84	18.1
	Valine D8	296.37	171.06	21	96	17.95
23	Isoluecine	302.1	171.03	21	96	22.04
24	Luecine	302.1	171.03	21	96	22.46
	Luecine D10	312.41	171.03	21	96	22.29
25	Phenylalanine	336.18	171	21	113	23
	Phenylalanine D2	338.37	171	21	113	22.97
26	Tryptophan	375.19	171	24	124	23.68
	Tryptophan D5	380.43	171	24	124	23.6

Table 1: SRM for amino acids analysed in the method.

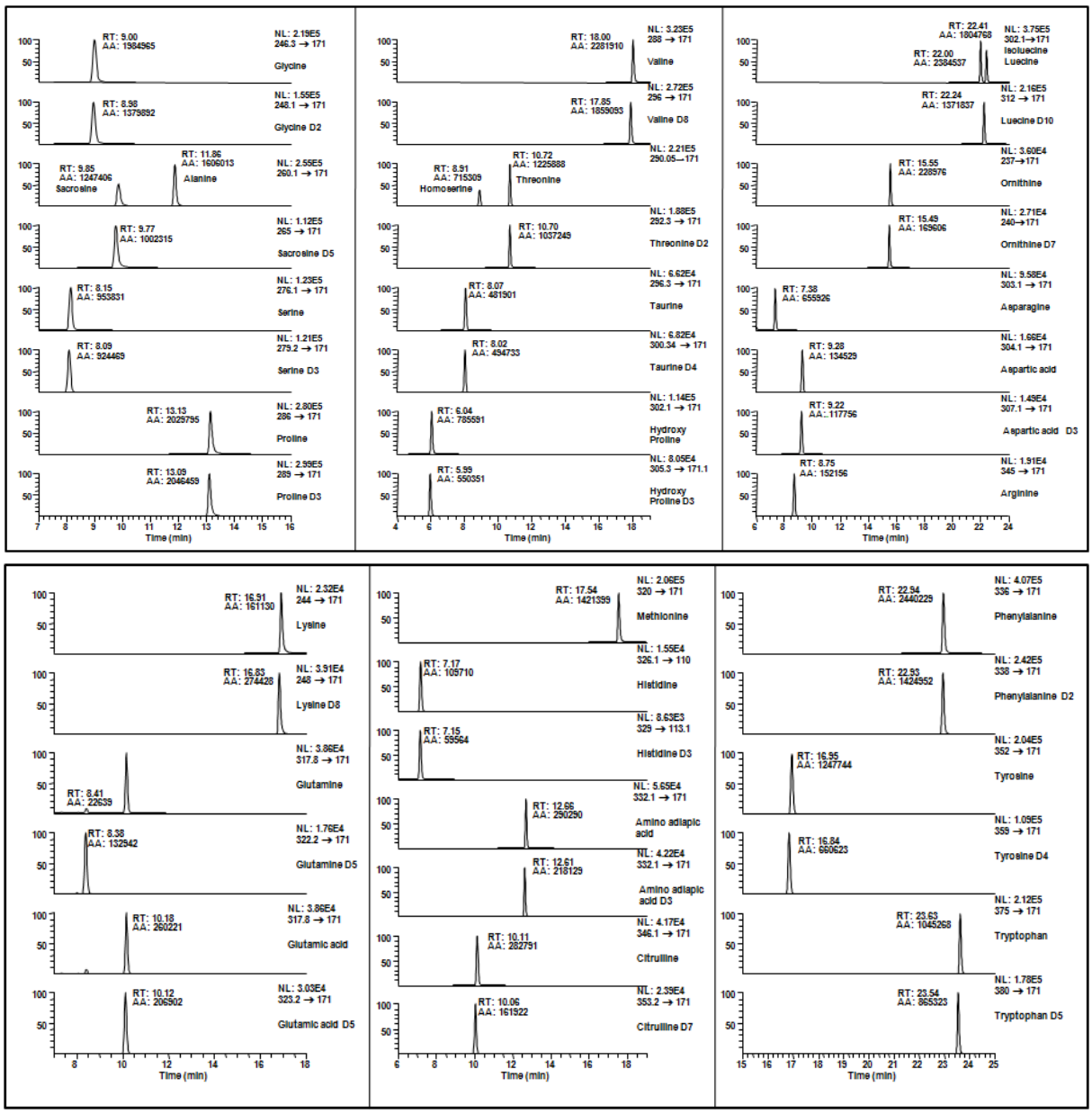

Figure 2: LC-MS/SRM chromatogram of 26 amino acids (10ng on column)