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

Metabolomics – Quantification of Neurotransmitters

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

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

Protocol:

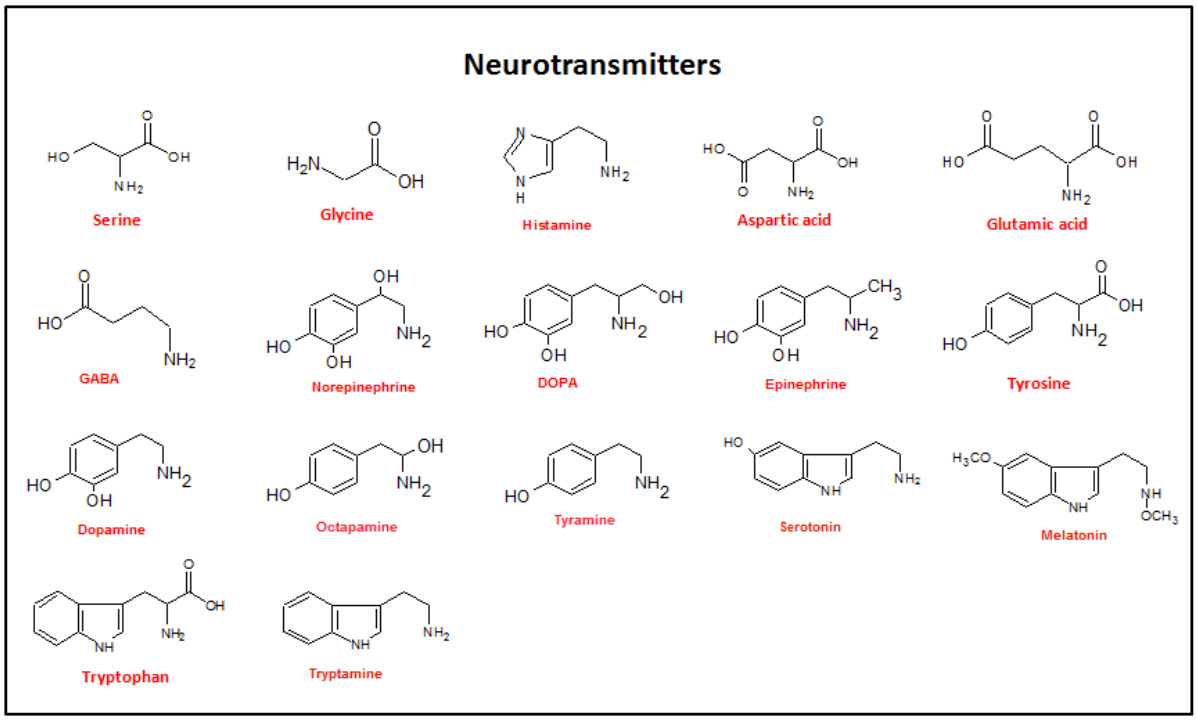

Figure 1: List of all seventeen neurotransmitters.

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 stock 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 Dopamine, DOPA, Epinephrine and Norepinephrine 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 guard column and 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 3.

	Name	Parent ion (m/z)	Product ion (m/z)	Collision Energy (CV)	S-lens Voltage	Retention time (min)
1	Serine	276.1	171.04	21	84	8.19
	Serine D3	279.2	171.1	20	92	8.16
2	Glycine	246.03	171.06	22	82	9.05
	Glycine D2	248.1	171.07	18	83	9.02
3	Histamine	282.37	138	19	73	9.18
	Histamine D4	286.37	142	19	73	9.13
4	Aspartic acid	304.1	171.04	21	98	9.31
	Aspartic acid D3	307.1	171.02	21	96	9.28
5	Glutamic acid	317.8	171	25	85	10.25
	Glutamic acid D5	323.26	171	21	85	10.19
6	Gaba	274.28	171	23	91	12.8
	Gaba D6	280.32	171	23	91	12.72
7	Nor epinephrine	340.34	171	24	93	13.58
	Nor epinephrine D6	346.38	171	24	93	13.51
8	Dopa	368.35	171	23	94	14.95
	Dopa D3	371.37	171	23	94	14.91
9	Epinephrine	354.37	171	28	73	14.95
	Epinephrine D6	360.4	171	28	73	14.81
10	Tyrosine	352.2	171	22	117	17.01
	Tyrosine D4	359.4	171	22	117	16.89
11	Dopamine	324.3	171	24	73	17.59
	Dopamine D4	328.37	171	24	73	17.49
12	Octapamine	324.17	171	24	91	15.26
13	Tyramine	308.36	171	22	93	20.28
14	Serotonin	347.38	171	27	93	19.35
	Serotonin D4	351.4	171	27	93	19.25
15	Melatonin	233.2	174.12	15	65	21.84
	Melatonin D4	237.2	178.13	15	57	21.71
16	Tryptophan	375.19	171	24	124	23.68
	Tryptophan D5	380.43	171	24	124	23.6
17	Tryptamine	331.38	171	27	91	26.05
	Tryptamine D4	335.4	171	27	91	25.98

Table 1: SRM for neurotransmitters analysed in the method.

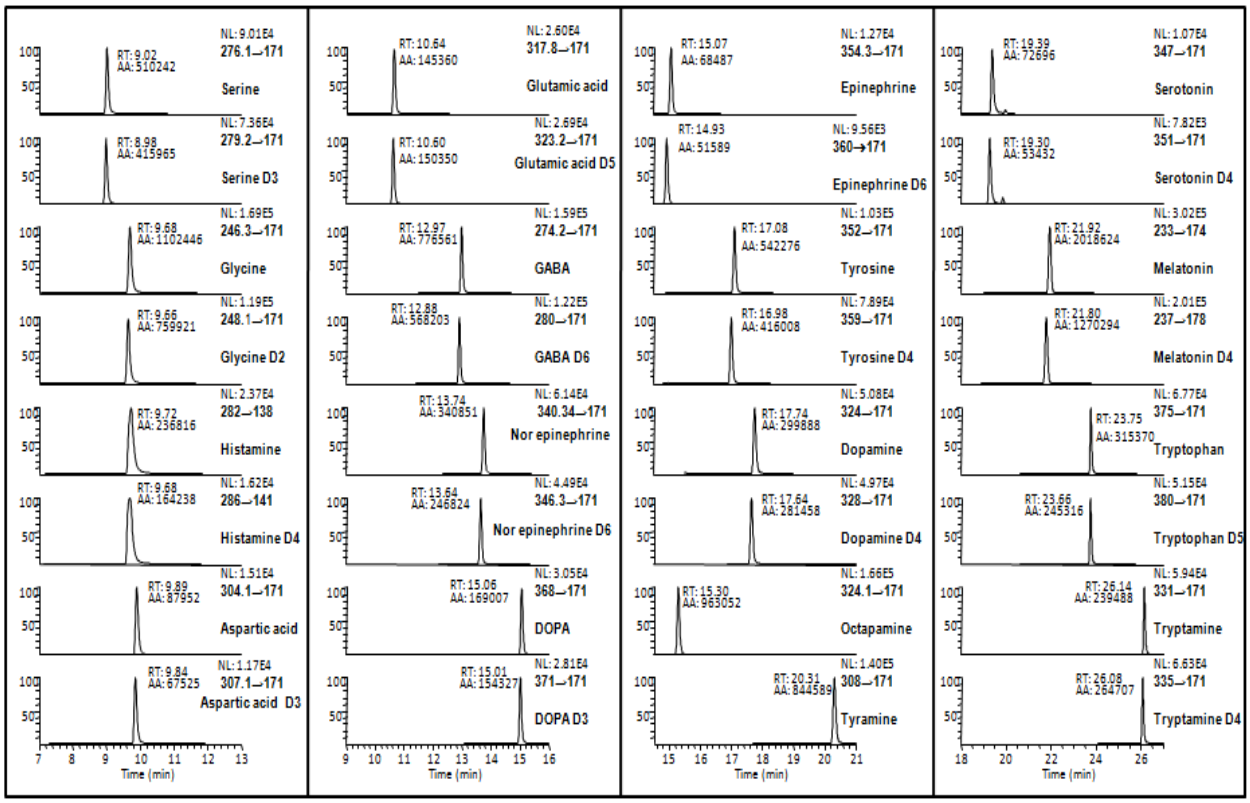

Figure 2: LC-MS/SRM chromatogram of 17 neurotransmitters.