Current Standard Operating Protocols (SOP), NCBS-CCAMP MS-Facility Metabolomics – Quantification of Olanzapine and its metabolites from sera

Purpose: To provide general guidelines for conducting the quantification of the psychotic drug olanzapine and its metabolites from sera using tandem triple quadrupole mass spectrometer.

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

Protocol:

A. Sample Preparation:

- Prepare the individual stock solutions (STDs) of each compound (~2mg/mL) either in 100% methanol (Stock A).

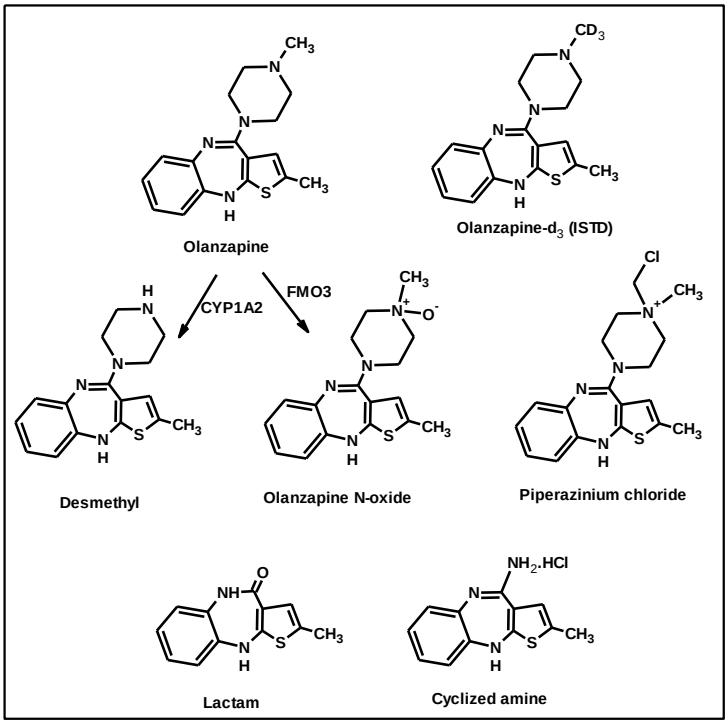

Figure 1: Structure of Olanzapine, Olanzapine-d_3 (ISTD), two metabolites and three of its impurity standards.

- Prepare the stock solutions of olanzapine-d3 (ISTD) (~1mg/mL) either in 100% methanol.
- Prepare 10 µg/mL stocks of both STDs and ISTDs in 100% methanol by taking the required amount from the individual stock.
- Prepare 1 µg/mL stock of both STDs and ISTDs in 100% methanol by diluting further from the 10 µg/mL stock.

- Prepare the seven point working standards (17 pg/mL to 1250 pg/mL) except lactam (0.085 ng/mL to 6.25 ng/mL) by serial diluting the 1 µg/mL stock in 200 µl of double charcoal stripped sera with the ISTD of all (1250 pg/mL).

B. Extraction of metabolites from sera:

- Extract metabolites from sera by precipitating proteins using 1 mL of methanol.
- Vortex the tubes in the thermo mixture (3 min, 1000 rpm) and centrifuge (5 min, 14,000 rpm).
- Transfer the supernatant to the fresh eppendorf tube and dry it using the speed vacuum.
- Reconstitute it in 50 µl of 0.5% acetonitrile (0.1% FA) and transfer this into the HPLC vial for the injection.

C. LC-SRM Analysis STDs and ISTDs:

- Equilibrate the C-18 column (Phenomenex 1.8 µ, 2.1 X 100 mm) with 2% acetonitrile.
- Use the mobile phase solvents A: water (0.1 % FA), B: Acetonitrile (0.1% FA) with the flow rate of 200 µL/min for the analysis.
- Set the following gradient (0 to 2 min-2 % B, 2 to 15 min- 2 to 40% B, 15 to 17 min-40 to 95% B, 17.1 to 20 min- 2% B) in the LC system.
- In the MS set the source parameters like spray voltage, 3500 V; source temperature, 100 °C; ion transfer capillary temperature, 280 °C; collision gas argon, S-lens voltage- as per table 1, sheath gas-18 and auxiliary gas-5; scan time-50 milli sec for each 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.

Table 1: MRM Table for Olanzapine and its Impurities.

Compound	Parent ion (m/z)	Product ion (m/z)	Collision Energy (CE)	S-lens voltage
Olanzapine	313.4	256.07	23	105
Olanzapine-d3	316.4	256.07	23	105
Cyclized amine	230.06	213.05	19	88
Lactam	231.2	214.03	20	106
Desmethyl	299.4	213.02	26	107
Olanzapine N-oxide	329.4	229.05	17	92
Piperazinium chloride	362.9	325.12	11	81

- Inject 10 µL of the working STDs (1.5 pg to 100 pg on column) for the analysis.

- The expected result for the STDs and ISTDs is shown in the following figure 2.

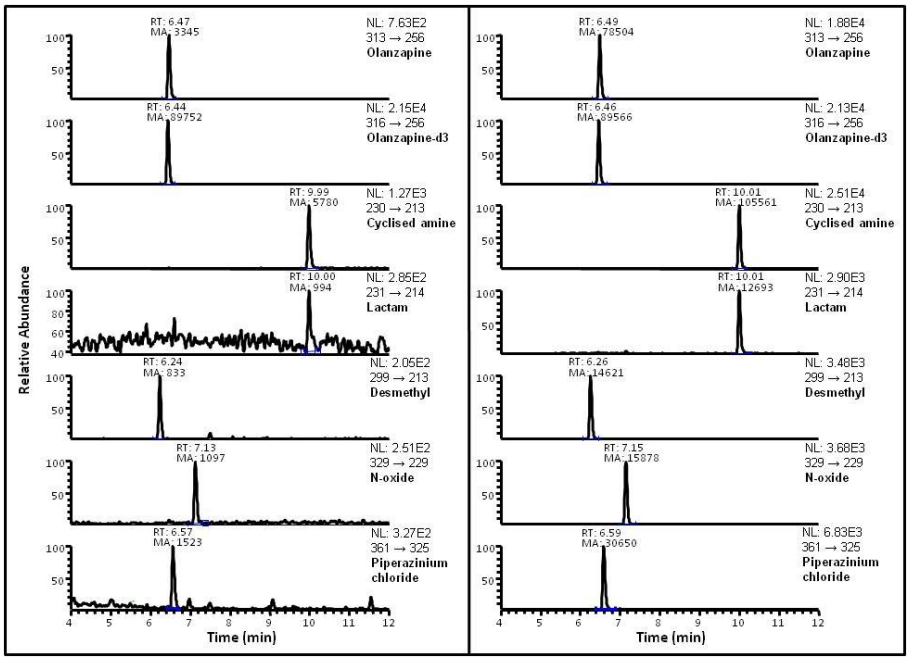

Figure 2: LC-MS/SRM chromatogram of Olanzapine and its impurity metabolites (LOQ and HQC).

- The constructed std curve for each metabolite is shown in the figure 3.

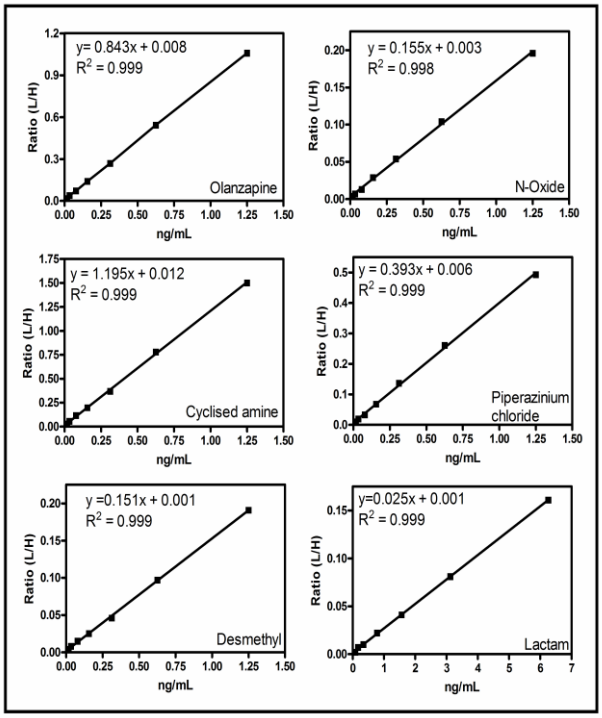

Figure 3: STD curves for Olanzapine and its impurity metabolites.