9/10/2012. Current Standard Operating Protocols (SOP), NCBS-CCAMP MS-Facility Metabolomics – Method Development and Validation

Purpose: To provide general guidelines for conducting routine tandem triple quadrupole mass spectrometer calibration.

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

Protocol:

Steps for the method development for specific metabolites to know the absolute quantification (STDs and ISTDs to be obtained from the companies)

(i) MS scan, MS/MS scan and picking the most intense ion for the SRM scan
(ii) Spiking the STD and ISTD to the matrix and extraction of metabolite from matrix (Sera, Saliva, Water, cell extract and Urine)
(iii) Detection of LOD and LOQ
(iv) Construction of STD curve
(v) Validation of the method (Inter & Intraday, recovery, accuracy and precision)

Example: Quantification of Melatonin from planarian extract.

(i). MS and MS/MS scan and picking the most intense ion for the SRM scan

- Prepare melatonin (100 ng/mL) solution by diluting the original stock solution (1mg/mL in methanol of melatonin) in 0.5% Acetonitrile (0.1% FA).
- Equilibrate the guard column cartridge with isocratic (30% A, 70% B) gradient with the flow rate of 100 µl/min.
- Transfer the sample (50 µl) into the HPLC vial and place it in the autosampler.
- Inject 10 µl of sample for the analysis.
- Do both full scan and product ion scan in the same method with two different scan events [MS san: 50 to 300 m/z, scan time 0.5 sec, MS/MS scan by selecting the parent ion (233 Da collision energy 15, with unit resolution in both quads)].
- The extracted molecular ion chromatogram and the corresponding spectrum for both MS and MS/MS were shown in the figure 1.
- The m/z of parent ion is 233 Da and the m/z of the most intense product ion is 174 Da.
- Select the transition from 233 → 174 for the SRM analysis.

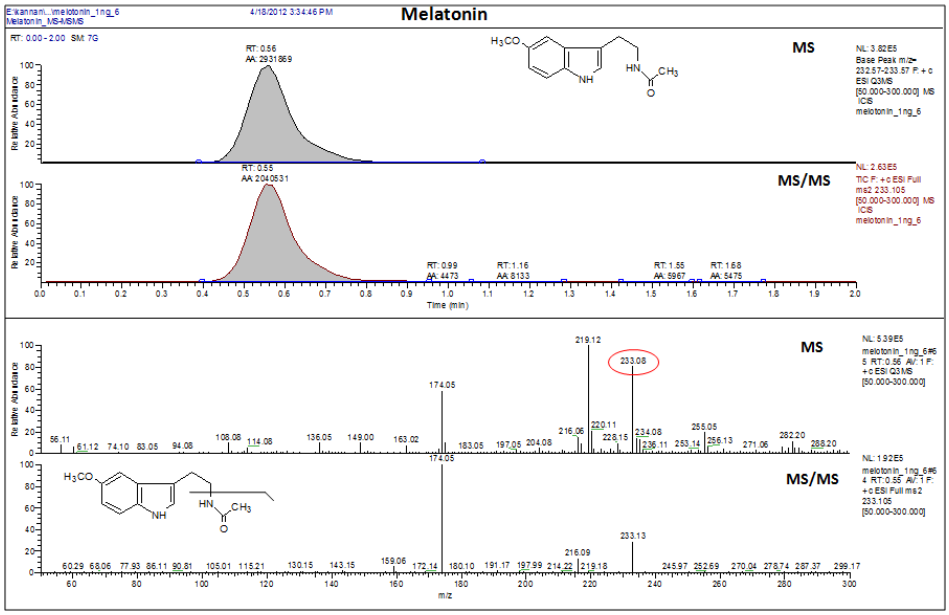

Figure1: Analysis of melatonin by MS and MS/MS experiment. Ten micro liter of melatonin (100 ng/mL) was injected. Analysis was done using C-18 Guard column cartridge with the flow rate of 100 µl/min and in isocratic mode [30% A (water with 0.1% FA), 70% B (ACN with 0.1% FA)].

(ii). Spiking the STD and ISTD to the matrix and extraction of metabolite from matrix

- Prepare the stock solution (STD and ISTD) of melatonin (~1 µg/mL) in 50% methanol (0.1% FA) by serial diluting the original stock solution (1mg/mL).

- Prepare the seven point working standards (0.78 ng/mL to 50 ng/mL) by serial diluting the 1 µg/mL stock in 200 µl acetone (0.1% FA) along with the ISTD of all (50ng/mL).

- Dry this in speed vacuum and reconstitute it in 50 µl of 0.5% acetonitrile (0.1% FA) and transfer this into the HPLC vial for the injection.

- The typical SRM chromatogram of melatonin from the lowest (0.78 ng/mL) and highest (50 ng/mL) and the STD corresponding STD curve was shown in the figure2.

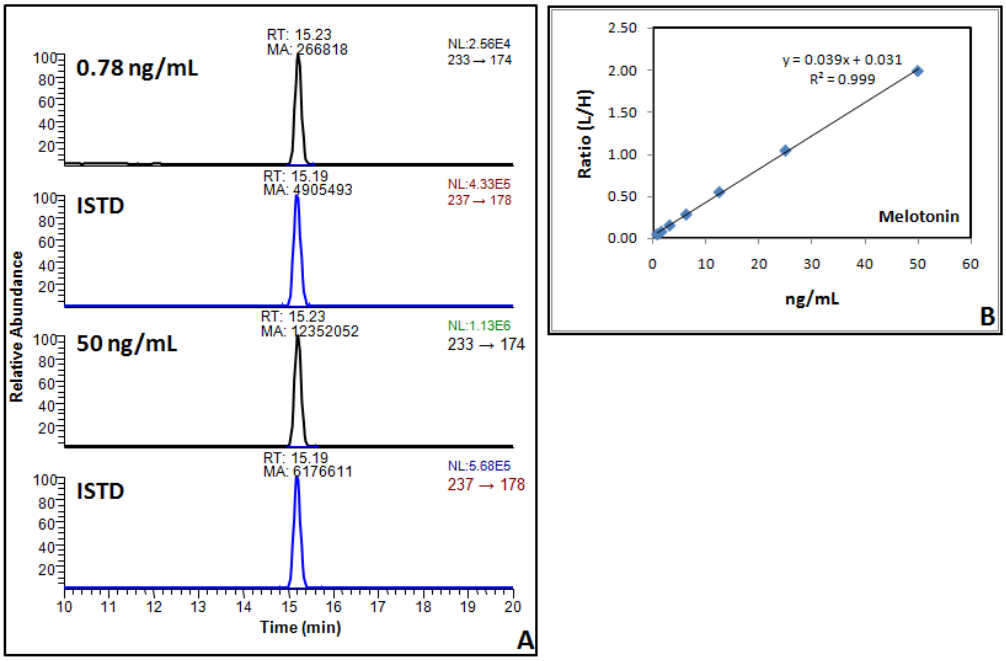

Figure 2: A) LC-SRM chromatogram of melatonin in the lowest and highest concentration. B) STD curve for melatonin.

(iii). Method Validation:

- Construct the STD curve along with the quality controls (LOQ, LQC, MQC and HQC) as shown in the table 1.
- Repeat this at least for three days to know the consistency of the method.
- Calculate the interday mean, coefficient of variation and accuracy as shown in the table 1.

Table1: Method validation for melatonin

	LOQ	LQC	MQC	HQC
Melatonin				
ng/mL	1.56	3.12	20.00	40.00
Interday mean	1.50	3.07	20.23	40.77
%CV (n=5)	7.03	7.23	4.98	2.51
Accuracy (%)	96.34	98.29	101.13	101.92