

Current Standard Operating Protocols (SOP), NCBS-CCAMP MS-Facility Metabolomics – Full Scan (MS) and production scan (MS/MS) analysis

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.

Full scan and product ion scan analysis: This can be done in three different ways

Through column: The samples are typically analyzed via LC/MS in positive ion mode, scanning from m/z 100-1500, on a C18 column. These methods can be modified to suit individual experimental needs.

Flow Injection with Guard Cartridge: In this case, samples will be injected (1 μ g/mL) directly along with flow of 100 μ l with the solvent composition of (30% A, 70% B) [A: Water (0.1% FA), B: Acetonitrile (0.1% FA)], Samples will be analyzed via LC/MS in positive ion mode, scanning from m/z 100-1500.

Direct Injection: In this case, samples will be injected directly using a syringe pump with the flow of 5 to 10 μ l/min. Sample concentration should be of 1 to 10 μ g/mL. Samples will be analyzed via LC/MS in positive ion mode, scanning from m/z 100-1500.

Example study: MS and MS/MS analysis of melatonin and adenosine.

Sample preparation:

- Prepare melatonin (100 ng/mL), adenosine (1 μ g/mL) solutions by diluting the original stock solution (1mg/mL in methanol of melatonin, 1mg/mL of in water (0.5% FA) of adenosine) 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 μ l/min.
- Transfer the sample (50 μ l) into the HPLC vial and place it in the autosampler.
- Inject 10 μ l of sample for the analysis.
- Do both full scan and product ion scan in the same method with two different scan events [MS scan: 50 to 300 m/z , scan time 0.5 sec, MS/MS scan by selecting the parent ion (233 and 268 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

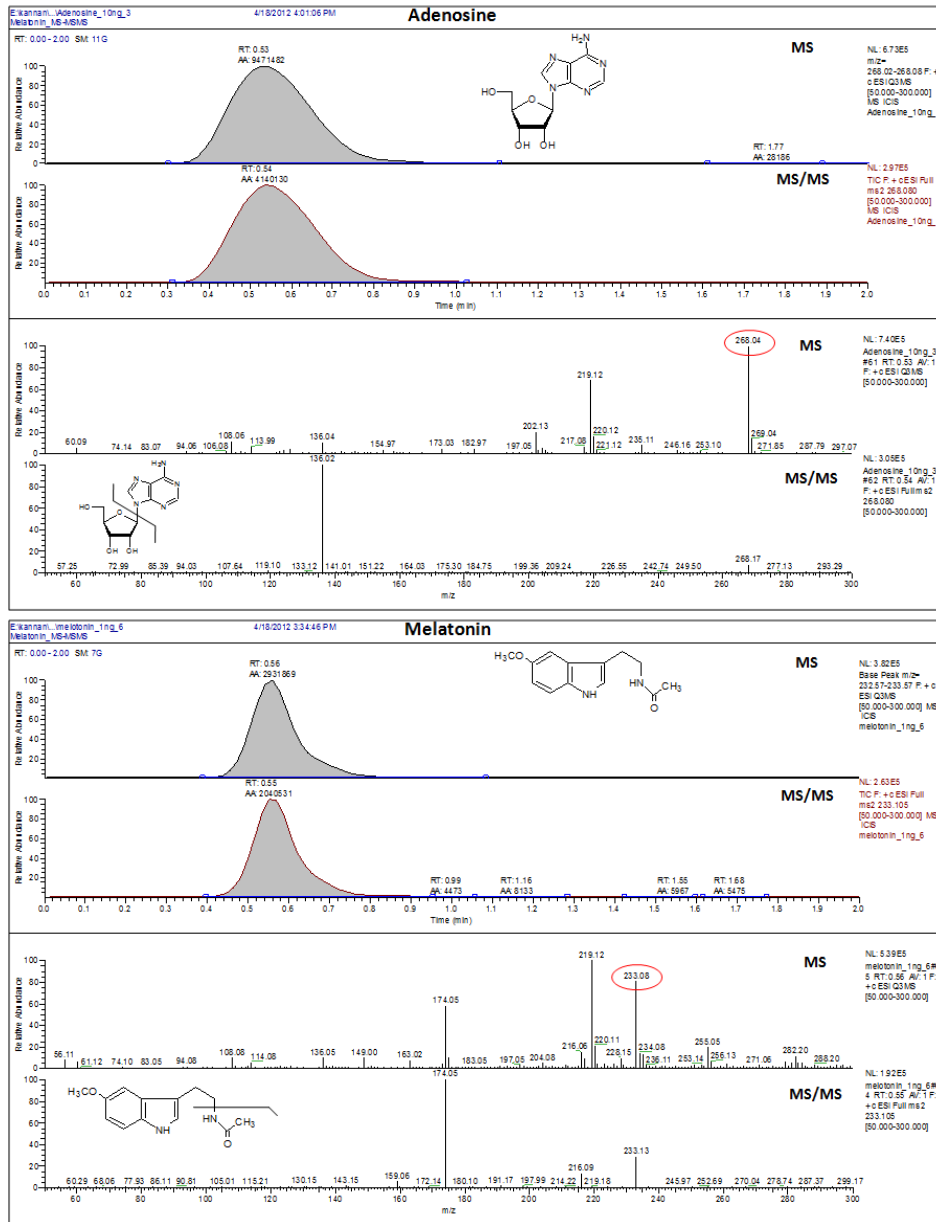


Figure1: Analysis of adenosine and melatonin by MS and MS/MS experiment. Ten micro liter of melatonin (100 ng/mL), Adenosine (1 µg/mL) were injected. Analysis was done using C-18 Guard column cartridge with the flow rate of 100 µl/min and in isocratic mode [30% A (water with 0.1% FA), 70% B (ACN with 0.1% FA)].