Cannabis Analyzer for Potency Quick Guide

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Shimadzu Scientific Instruments is not condoning the use of recreational nor medical marijuana, we are merely providing a market summary of the cannabis testing industry.

Cannabis Analyzer for Potency is used to determine the cannabinoid content of flowers, leaf and trim, concentrates, edibles and other samples. Up to 11 (or 10) cannabinoids, Δ9-THCA, Δ9-THC, CBDA, CBD, CBN, CBC, CBGA, CBG, CBDV, THCV and Δ8-THC can be analyzed in a single chromatographic run with a cycle time of approximately 10 (or 8) minutes.

Preparation for Analysis

1. **Mobile Phase** – Pre-modified mobile phase specific to the following methods may be purchased directly from Shimadzu.

For High-Throughput method:

- Honeywell/Jade Mobile Phase for HT Method (P/N: 220-91394-80; UHPLC grade)
  - Mobile Phase A: 0.1% Formic acid in water
  - Mobile Phase B: 0.1% Formic acid in acetonitrile
  - Rinse Solution: 0.1% Formic acid in acetonitrile

For High-Sensitivity method:

- Honeywell/Jade Mobile Phase for HS Method (P/N: 220-91394-81; UHPLC grade)
  - Mobile Phase A: 0.085% Phosphoric acid in water
  - Mobile Phase B: 0.085% Phosphoric acid in acetonitrile
  - Rinse Solution: 0.085% Phosphoric acid in acetonitrile

For High-Resolution method

- Honeywell/Jade Mobile Phase for HR Method (P/N: 220-91394-82; UHPLC grade)
  - Mobile Phase A: 0.085% Phosphoric acid in water
  - Mobile Phase B: 0.085% Phosphoric acid in methanol
  - Rinse Solution: 0.085% Phosphoric acid in methanol

Only HPLC or UHPLC grade water and acetonitrile should be used for mobile phase.

Standards Mixture

Certified Reference Material (CRM) standards may be purchased directly from Shimadzu, shown below.

1. **Prepare Standards**
   a) Prepare the Standards per the method package the customer has purchased.
      - Check that the concentrations listed below are the correct concentrations on the standard vials.

For High-Throughput method:

- Analytical Standards – 10 Components (P/N: 220-91239-20)

#	Compounds	Conc. (mg/L)
1	CBDV	250
2	CBDA	250
3	CBGA	250
4	CBG	250
5	CBD	250
6	CBN	250
7	Δ9-THC	250
8	Δ8-THC	250
9	CBC	250
10	Δ9-THCA	250

- Standard Count: 4
- Calibration range: 5-100 mg/L

No.	Sample Name	Sample ID
1	10 Standards mixture	5.0 ppm
2	10 Standards mixture	10.0 ppm
3	10 Standards mixture	50.0 ppm
4	10 Standards mixture	100.0 ppm

100 mg/L standard mixture (10 components)

1. Transfer 400 µL of Analytical Standards (P/N: 220-91239-20 or 220-91239-21) to an HPLC vial and add 600 µL of methanol.
2. Vortex for 30 seconds.

50 mg/L standard mixture (10 components)

1. Transfer 500 µL of 100 mg/L standard mixture to an HPLC vial and add 500 µL of methanol

2. Vortex for 30 seconds.

10 mg/L standard mixture (10 components)

1. Transfer 200 μL of 50 mg/L standard mixture to an HPLC vial and add 800 μL of methanol.
2. Vortex for 30 seconds.

5 mg/L standard mixture (10 components)

1. Transfer 500 μL of 10 mg/L standard mixture to an HPLC vial and add 500 μL of methanol.
2. Vortex for 30 seconds.

For High-Sensitivity method:

- Analytical Standards – 11 Components (P/N: 220-91239-21)

#	Compounds	Conc. (mg/L)
1	CBDV	250
2	CBDA	250
3	CBGA	250
4	CBG	250
5	CBD	250
6	THCV	250
7	CBN	250
8	Δ9-THC	250
9	Δ8-THC	250
10	CBC	250
11	Δ9-THCA	250

- Standard Count: 6
- Calibration range: 0.5 – 100 mg/L

No.	Sample Name	Sample ID
1	11 Standards mixture	0.5 ppm
2	11 Standards mixture	1.0 ppm
3	11 Standards mixture	5.0 ppm
4	11 Standards mixture	10.0 ppm
5	11 Standards mixture	50.0 ppm
6	11 Standards mixture	100.0 ppm

100.0 mg/L standard mixture (11 components)

1. Transfer each 400 μL of the standard mixture to an HPLC 1.5mL vial and add 600 μL of methanol.
2. Vortex for 30 seconds.

50.0 mg/L standard mixture (11 components)

1. Transfer 500 μL of 100.0 mg/L standard mixture to an HPLC vial and add 500 μL of methanol
2. Vortex for 30 seconds.

10.0 mg/L standard mixture (11 components)

1. Transfer 200 μL of 50.0 mg/L standard mixture to an HPLC vial and add 800 μL of methanol.

2. Vortex for 30 seconds.

5.0 mg/L standard mixture (11 components)

1. Transfer 500 µL of 10.0 mg/L standard mixture to an HPLC vial and add 500 µL of methanol.
2. Vortex for 30 seconds.

1.0 mg/L standard mixture (11 components)

1. Transfer 200 µL of 5.0 mg/L standard mixture to an HPLC vial and add 800 µL of methanol.
2. Vortex for 30 seconds.

0.5 mg/L standard mixture (11 components)

1. Transfer 500 µL of 1.0 mg/L standard mixture to an HPLC vial and add 500 µL of methanol.
2. Vortex for 30 seconds.

High-Resolution method:

- Analytical Standards – 11 Components (P/N: 220-91239-21)

#	Compounds	Conc. (mg/L)
1	CBDV	250
2	CBDA	250
3	CBGA	250
4	CBG	250
5	CBD	250
6	THCV	250
7	CBN	250
8	Δ9-THC	250
9	Δ8-THC	250
10	CBC	250
11	Δ9-THCA	250

- Standard Count: 7
- Calibration range: 0.5 – 250 mg/L

No.	Sample Name	Sample ID
1	11 Standards mixture	0.5 ppm
2	11 Standards mixture	1.0 ppm
3	11 Standards mixture	5.0 ppm
4	11 Standards mixture	10.0 ppm
5	11 Standards mixture	50.0 ppm
6	11 Standards mixture	100.0 ppm
7	4 Standards mixture	250 ppm

250 mg/L standard mixture (4 components)

1. Transfer 500 µL of 11 component standard mixture to an HPLC vial.
2. Vortex for 30 seconds.

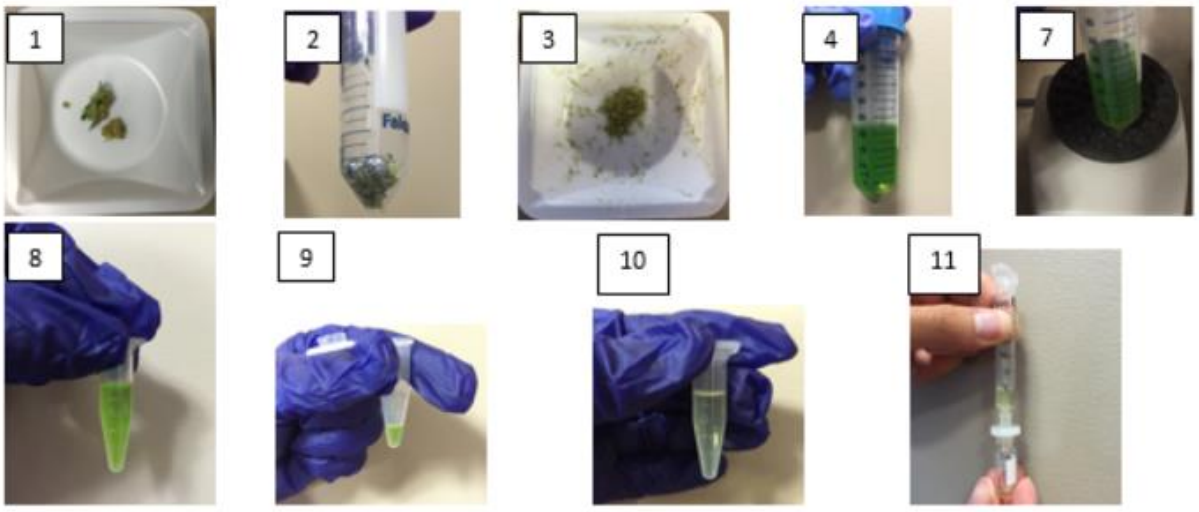
Standards 1-6 (concentrations 0.5 – 100 mg/L) follow High Sensitivity Standard preparation method above.

Unknown Sample Preparation

Cannabis Flower - Preparation procedures for cannabis flower are described below.

- This procedure makes uses a 2010 Geno/Grinder (SPEX SamplePrep, LLC) and shaker to powderize the sample.

1. Weigh 200 mg into a 50 mL centrifuge tube.
2. Transfer two 9.5 mm O.D. steel balls into the tube.
3. Shake at 1000 rpm for 1 minute using a 2010 Geno/Grinder.
4. Add 20 mL of methanol to the tube. (Extraction Vol. 20 mL)
5. Shake at 1000 rpm for 1 minute using a 2010 Geno/Grinder.
6. Wait 15 minutes.
7. Mix using a vortex mixer for 1 minute.
8. Transfer 1 mL of solvent into a 1.5 mL microtube, and centrifuge at 3000 rpm for 5 minutes.
9. Transfer 50 μL of supernatant into a new 1.5 mL microtube.
10. Add 950 μL of methanol and vortex for 30 seconds. (20 times dilution, Dilution Factor: 20)
11. Filter using a 0.45 μm syringe filter, and transfer into a 1.5 mL HPLC vial.
12. The sample is now ready for analysis using the Analyzer.



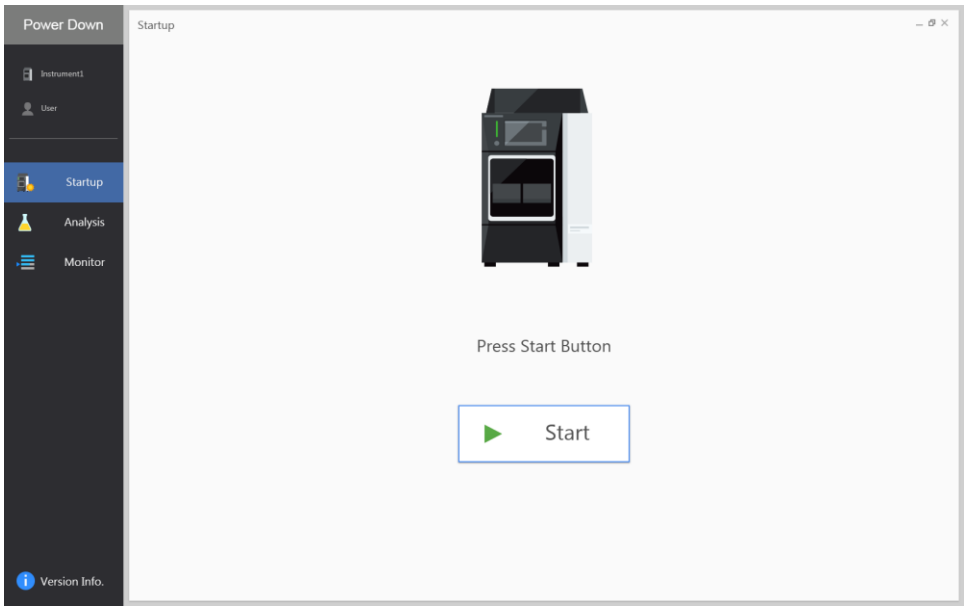
Analyzer Start Up

Note: This document presumes that Shimadzu LabSolutions software (Version 592 or newer) has first been installed and that the Cannabis Analyzer for Potency Version 1.1.0 software has been installed.

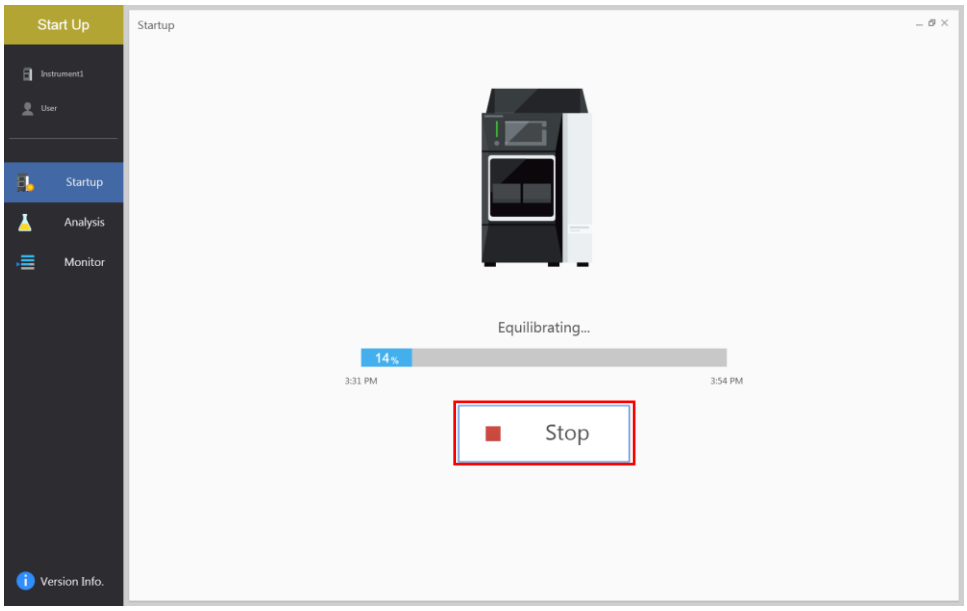
- Data and method files are located in the Cannabis Analyzer folder on the computer
  - Method files - in the C:\LabSolutions\Data\CannabisAnalyzer*methodfolder* folder.
  - Data files - located in a new folder automatically created each day (labelled as the date) to receive the data files generated.

a) Double click the Cannabis Analyzer for Potency icon on the computer desktop. The instrument selection window will open.
b) If multiple Analyzers have been configured, they will all appear as separate instruments.
c) Double click on the appropriate instrument.

d) The Start Up screen will appear.
e) Click the **START** button, shown below.



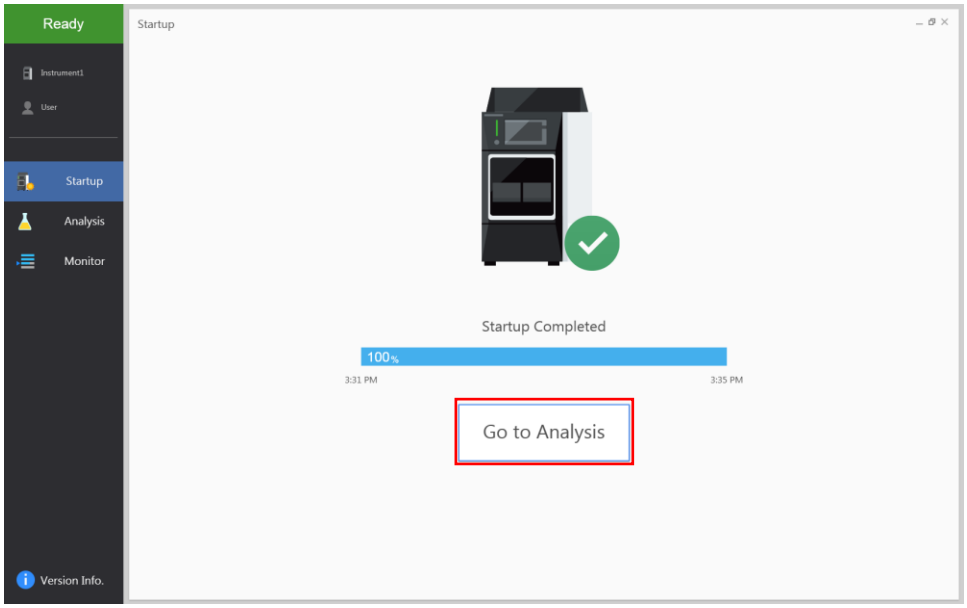
f) The instrument will initialize, which will last several minutes.
   - The progress of startup will appear on the screen along with messages to indicate which modules are being initialized and tested.



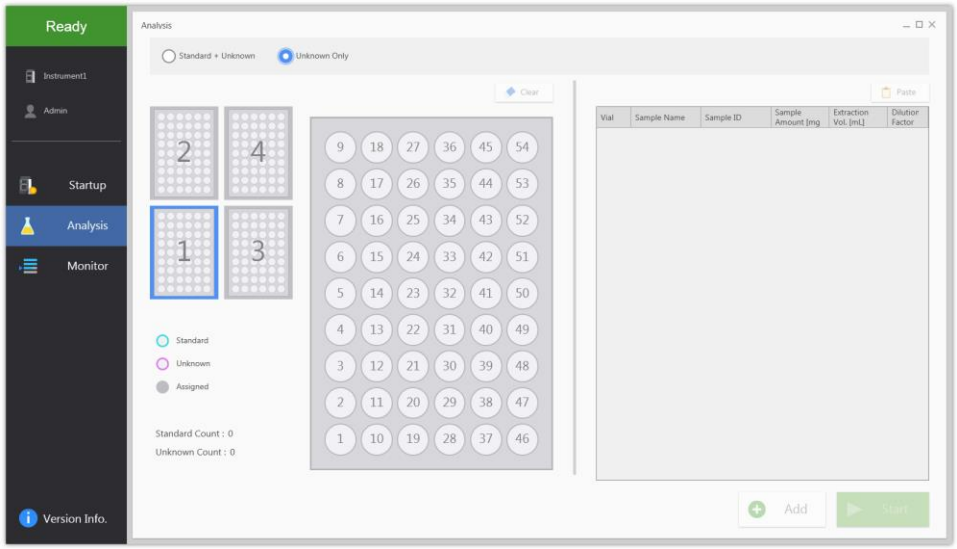
g) When the initialization sequence is complete you will be prompted for a password on the instrument display panel.
   - Enter *00000* (five zeroes) from the instrument touch panel and then press “OK”.

Data Acquisition

a) When Startup has completed
b) Click on the GO TO ANALYSIS button.



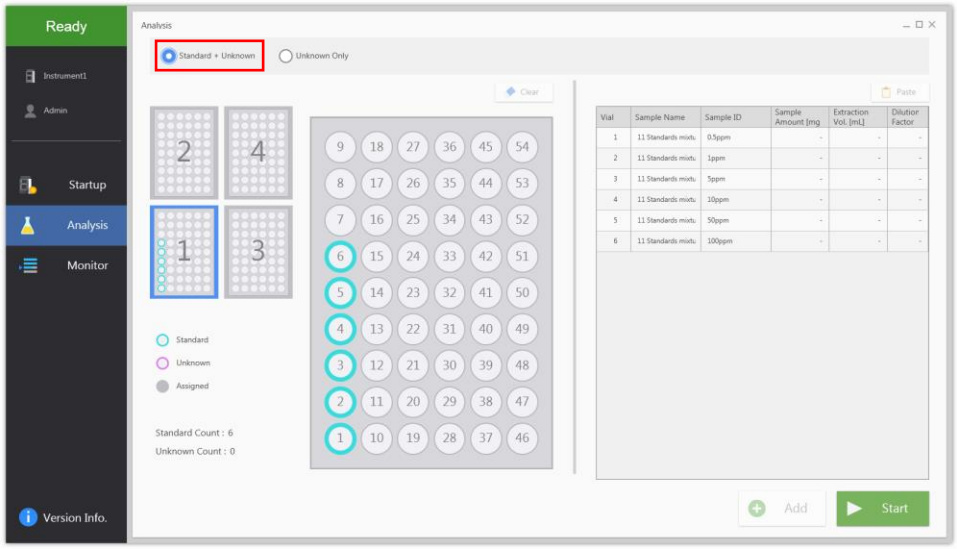
c) Analysis section of the Cannabis Analyzer for Potency screen will appear



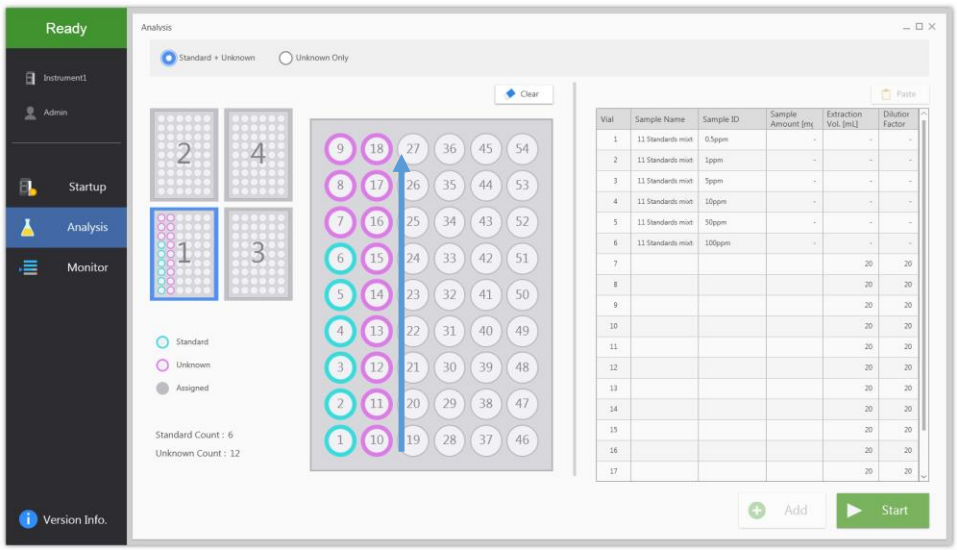
d) At the top of the screen are two modes of operation, “Standard + Unknown” and “Unknown Only”.
- (Standard + Unknown) is used when you want to create a standard curve for calibration using a set of standards followed by a series of unknown samples.
- (Unknown Only) is for running only unknown samples with the last calibration that was performed –a new calibration will not be created.

e) In the case of “Standard + Unknown” and place the six previously prepared standard mixtures on the sample rack (Tray 1, vials # 1 – 6), indicated by the blue circles.
   - Place the vials in increasing order of concentration, with vial #1 being the low standard and vial #6 being the high standard.

NOTE: According to the method, the first six vial positions on the tray graphic will indicate the standards. These are circled in blue as shown below. Notice that the standards are also labeled with a “Sample Name” and “Sample ID” on the Sample Information Table on the right side of the screen.



f) Drag the mouse across the vial positions starting at #7 through the number of unknown samples you wish to analyze, as shown below.
   - Notice in the screen shot below that unknown samples from 7 to 18 have been selected and are added to the Sample Information Table to the right. These are highlighted in pink to indicate they are unknowns.

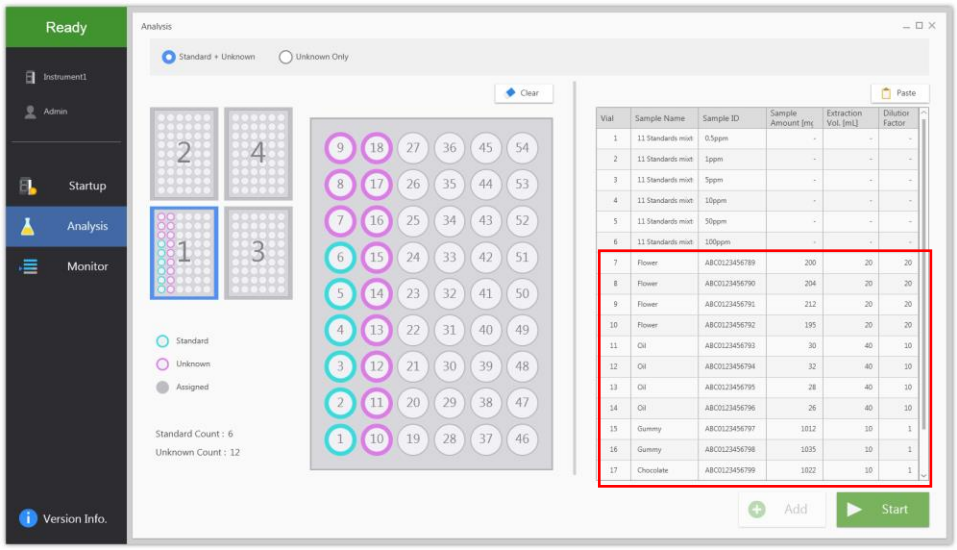


g) Enter the “Sample Name” and “Sample ID” on the Sample Information Table.

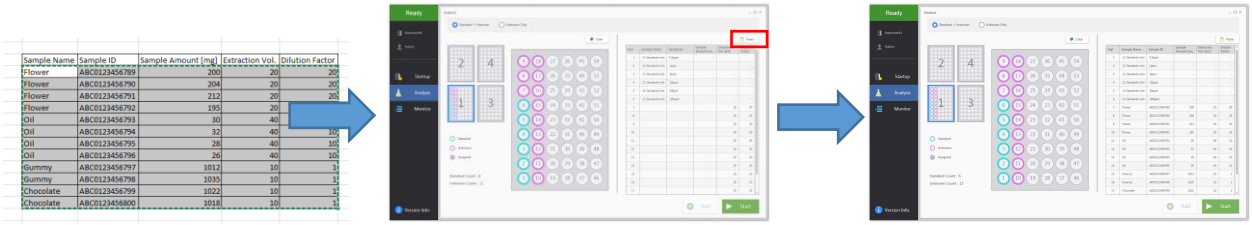
h) According to the sample preparation:

- Sample Amount – Enter the dry weight of sample (mg)
- Extraction Vol. – Enter the extracted volume (mL) (From Step 4 of sample prep)
- Dilution Factor – Enter the dilution factor used. (From Step 10 of sample prep)

Note: The Extraction Vol. and Dilution Factor are each set by default to 20 base on the sample preparation method provided, but they can be changed according to sample preparation.

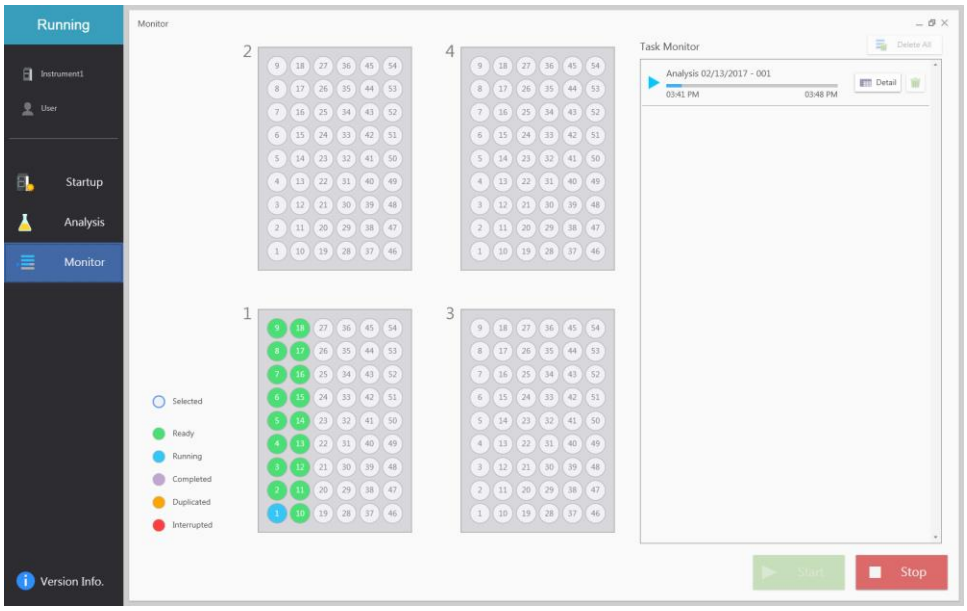


i) You can also copy and paste the “Sample Name” and “Sample ID” from Microsoft® Excel®.



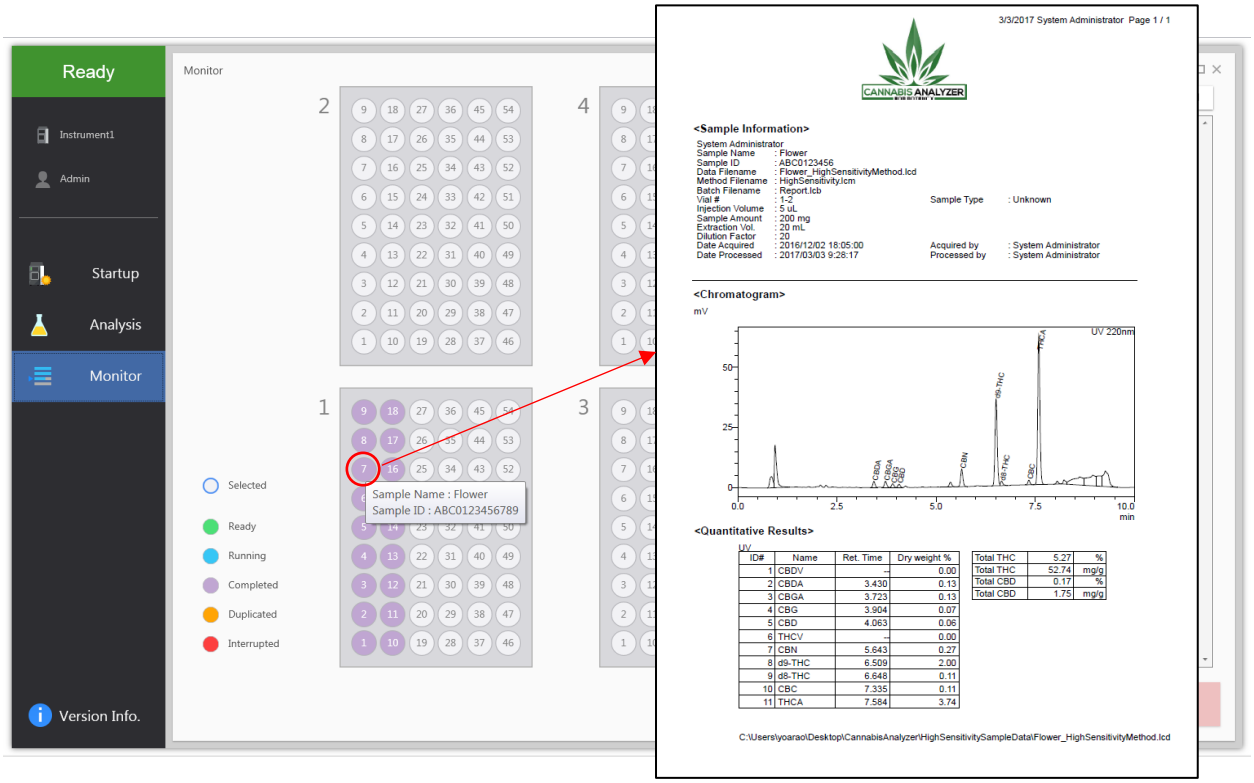
j) Click the START button on the lower right to start analysis.

- The screen will change to the Monitor view, as shown below.
- Notice that, in the top left corner, the status indicator has changed from READY to RUNNING.

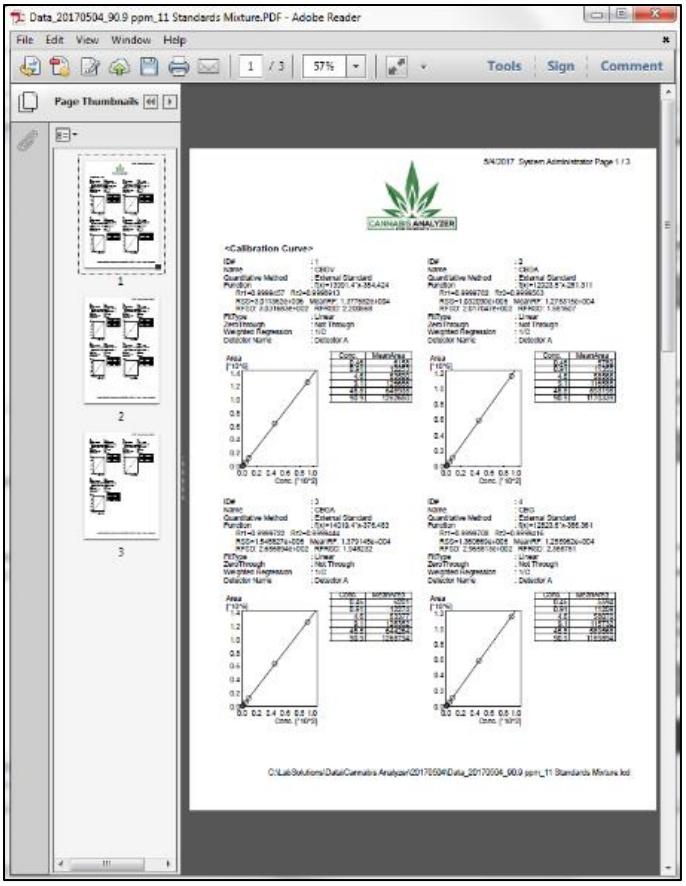


Note: that the Monitor view shows the samples in the tray with a color according to their status in the run queue:

- Purple - completed samples
- Blue - currently running sample
- Green - samples that are ready and waiting.
- The estimated time of completion for the entire “batch” is shown on the right side of the screen in the Task Monitor.



k) Double click the completed vial position to reveal the report.
   - Unknown – Quantitation report will display as seen above
     - The report reflects - Dry Weight % for each target compound, potency calculations for Total THC and Total CBD in % and mg/g.
   - Standard – Calibration report will display for R^2 verification
     - Use the final standard and R^2 should be above 0.999.



l) Reports are stored in the method folder in the date analyzed
   - Example: