

## Standard Operating Procedure for Hazardous Chemicals

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**Building and rooms:** Davis Life Sciences Bldg, Lab B310

<b>Chemical(s)</b>	Methanol, Acetonitrile, Formic Acid
<b>Process</b>	<b>Soluble Phenolic HPLC-MS-TOF Profiling Analysis</b>
<b>Specific Hazards</b> <i>refer to MSDSs for more detailed information</i>	<u>Methanol</u> is flammable, and may be harmful upon ingestion. <u>Acetonitrile</u> is flammable and harmful upon inhalation, eye and skin absorption and ingestion, and may cause skin and eye irritation. <u>Formic acid</u> is harmful and causes irritation upon inhalation, ingestion, and eye contact.
<b>Personal protective equipment</b>	Must wear 3-5 mil nitrile gloves. Chemical safety goggles and lab coat should be worn when splash potential exist
<b>Engineering/ventilation controls</b>	All operations involving acetonitrile must be done in a chemical fume hood.
<b>Special handling procedures and storage requirements</b>	Store <u>acetonitrile and methanol</u> in the flammable cabinet underneath the chemical hood in Rm B310.
<b>Spill and accident procedures</b> <i>for hazardous chemicals only</i>	<u>Skin exposure:</u> Rinse affected skin with plenty of water while removing contaminated clothing/shoes. Rinse for > 15 minutes. Treat acetonitrile exposure as cyanide poisoning. <u>Eye exposure:</u> Wash eyes for > 15 minutes. For both cases, seek medical attention immediately.
	<u>Small</u> (< 2L): Absorb with vermiculite or spill pads and transfer absorbed material to a closed container. Label and date as hazardous waste for disposal. Notify PIs.
	<u>Large</u> (> 2L): Evacuate the room, notify PIs and call 123 to request emergency spill assistance from Occupational Safety & Health Ser.
<b>Waste disposal</b>	Acetonitrile and methanol waste must be collected and labeled as hazardous wastes according to the SOP for Hazardous Waste Disposal. HPLC sample vials may be collected together inside two ziplock plastic bags, and then packed in a cardboard for Hazardous Waste pickup.
<b>Special approval</b>	No special authorization needed after SOP training & reading MSDSs.
<b>Prepared by</b>	Name/date: BA Babst, 10/02/2009.
<b>Reviewed by</b>	Name/date:

## Phenolic Profiling HPLC-MS-TOF Method

### Solutions and Reagents needed

**400  $\mu$ M  $^{13}\text{C}_6$ -cinnamic acid, 700 $\mu$ M D<sub>5</sub>-Benzoic acid, and 1.3mM resorcinol in 100% MeOH,** (to prepare the MeOH with internal standards, first prepare standards individually as 100mM stocks in 50:50 H<sub>2</sub>O:MeOH. Then prepare an adequate amount of MeOH with the appropriate concentrations of internal standards. Keep the stocks frozen! Return stocks to freezer immediately after use.)

**Mobile phase 1 (aqueous): 97% dd-H<sub>2</sub>O  
3% Acetonitrile  
0.1% formic acid**

**Mobile phase 2 (organic): 97% Acetonitrile  
3% dd-H<sub>2</sub>O  
0.1% formic acid**

**\*\*\*Do not attempt to operate HPLC-MS-TOF without prior instruction. Improper use may result in damage to instrumentation that is VERY EXPENSIVE to repair/replace.**

**Precautions:** Gloves and eye protection should be worn when handling chemicals.

### **Procedure:**

1. Analyze ground freeze-dried tissue powders: Weigh 10 (+0.5) mg powder into 1.5ml eppendorf tube and record weight for final calculations. Add 500ul ice-cold 100% methanol containing the appropriate internal standards (400 $\mu$ M  $^{13}\text{C}_6$ -cinnamic acid, 700 $\mu$ M D<sub>5</sub>-Benzoic acid, and 1.3mM resorcinol). Sonicate immediately in ice-cold water for 5 mins (7-10 mins may be required for coarser ground material).
2. Centrifuge the powder residue to the bottom of the tube, and carefully transfer the pigment-rich methanol to a new clean 1.5 -ml eppendorf tube. If there is still significant green color in the pellet, repeat the methanol sonication step and pool the two methanol extracts.
3. Transfer ~100ul of the MeOH extract to an HPLC sample vial with glass insert (no less than 50ul), and seal with the appropriate screw cap or crimp caps. Keep MeOH extracts cold (4°C) at all times. If you need to delay analysis, samples will be okay in -80°C for a few days.
4. Make sure you have enough mobile phase to run all of your samples (17ml per run plus ~100ml for start and stop). Make more if needed. **DO NOT LET COLUMN DRY!** Start up HPLC and MS, flush/equilibrate column with appropriate mobile phase, and calibrate mass spec. Set autosampler chiller to 4°C at least 30mins before placing samples. **DO NOT OPERATE HPLC-MS WITHOUT PROPER TRAINING!**
5. Set up "worklist" with sample, method, and file name. Make a folder with your name located in "D:\data". Within your folder, make a folder for today's run, named with the year, month, day, and optionally some word or two of description. For example, "2009-09-30\_Nstress\_Lpi5." For regular PG analysis and phenolic profiling use the method: "Ben\_SAGT-Assay\_Neg\_mod-6\_RR" If you think you need to use a different method speak to CJ or Scott AND Ben before proceeding. **ALWAYS start with 2-3 blank runs to be sure**

everything is running properly, and to equilibrate the column. Without this, automated profiling will become considerably more difficult or impossible. At the end of your worklist, be sure to include a blank run to clean the column, and a final run with the shutdown method, to leave the column in 50:50 H<sub>2</sub>O:MeOH (make sure there is H<sub>2</sub>O and MeOH in the #2 mobile phase bottles).

6. Place samples in autosampler so the samples match with vial #s in your worklist.
7. Run. For stem or root samples test a sample first, and compare to a leaf chromatogram. You may need to increase the injection volume to 2 or 3 ul to get good signal.
8. When your run is complete. Open up your data files in Masshunter Qual software and make sure everything looks okay. You might see one run where the retention times are off, or maybe an anomolous peak in one sample. If you check right away, you can run any weird samples again, so you are sure whether there is something biologically weird, or if it was just some analytical anomaly that can be discarded.
9. REMOVE YOUR SAMPLES FROM THE AUTOSAMPLER. Other people will need to use the machine. For most purposes, you will not need to save the samples. The vials can be disposed intact in double plastic bags (must both be ziplock).

### **LC separation method**

Setup plumbing to bypass mixer on LC pump. Minimize pre- and especially post-column volume.

Column: Agilent rapid res HT 4.6 x 50mm 1.8micron rated up to 600bar

Standard injection for leaf extracts: 1ul

Flow rate: 1ml/min

Time	%B
0	3
1	3
3	17
5	17
9	60
11	98

Stop at 13 mins., and have 3mins Post-time.

**MS parameters**

Negative mode

ESI: gas temp 350, drying gas 13, nebulizer 60 psi

VCAP 3500

Fragmentor 125