Combi*Flash*® Torrent™ Verification

Using Torrent Verification Kit (60-5244-128)

TELEDYNE ISCO
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Instruction Sheet #60-5243-158
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Background

This procedure is used to verify proper operation of Teledyne ISCO's Combi*Flash* Torrent module. The procedure can detect errors in solvent gradient formation and detection sensitivity. Proper alignment of the fraction collector is evidenced by the collection of peaks.

This system verification assumes that you are familiar with the operation of the Combi*Flash* Torrent module. If concerns arise about operating the module, consult the user manual.

Universal Verification Kit Description

Each vial contains 50 mg of Phenacetin and 200 mg N-Benzylbenzamide. The system may be verified with either normal or reverse phase solvent systems. For Torrent AQ instructions, see Section 2 of this document.

Section 1: Normal Phase

Required Apparatus and Reagents

- Combi*Flash* Torrent module with adapters installed for small column sizes.
- 330 gram RediSep® silica gel column (69-2203-330)
- Hexane and ethyl acetate, minimum A.C.S. reagent grade (or equivalent)
- Universal Verification Kit (P/N 60-5234-317)

Purging

Before proceeding, the Combi*Flash* Torrent module should be ready to run with hexane (A solvent) and ethyl acetate (B solvent). If the system is using a different solvent system, or if this is the first run for a new system, the internal plumbing must be purged and reprimed.

Note

The Combi*Flash* Torrent module is shipped from the factory with isopropyl alcohol (IPA) in the internal solvent lines.

To purge the module:

1. Connect the solid load cartridge tubing to the Sample Injection Port.
2. Insert a column bypass tube.

CAUTION

If the current solvent system is not miscible with hexane or ethyl acetate, perform steps 3 through 9 twice. On the first set, substitute hexane and ethyl acetate (step 3) with intermediate solvents. Then use hexane and ethyl acetate for the second set.

3. Ensure that the Solvent A inlet line is receiving hexane, and Solvent B inlet lines are receiving ethyl acetate.
4. From the menu, select the **Tools>Manual Control** to open the **Manual Control** window.
5. Select the Through **Column & Cartridge Valve Position** setting.
6. Touch the **Prime B** button. The solvent pump will start.
7. After at least 400 mL of solvent has been pumped, touch the **Stop** button.
8. Touch the **Prime A** button. The solvent pump will start.
9. After at least 400 mL of solvent has been pumped, touch the Stop button.
10. Close the **Manual Control** window.
11. After the pressure gauge on the touch screen has returned to 0 psi, remove the column bypass tube and disconnect the solid load cartridge tubing from the Sample Injection Port.

Note

Because an air purge was not performed, some solvent may drain when removing the column bypass tube.

The Combi*Flash* Torrent module is now ready for the Verification Procedure.

Verification Procedure

1. Connect the Liquid Injection Accessory from the accessory kit to the Sample Injection Port.
2. Load the 330 gram column on the module. Rotate the column so the label faces the RFID antenna.
3. Use the default method for the 330 gram column.
4. Optionally, in the Peak Collection section of the display, select the **Peaks** option.
5. On the Main screen, touch the **Play** button. This opens the **Run Requirements** window.

   a. Select Liquid for the Sample Loading Technique.

   Select the Start Rack and **Start Tube** number for the fraction collector. If using the Fractionation Valve, the set of containers is considered a rack. The **Start Tube** number identifies which of the six valve positions to start from.

   b. Review the Solvent Requirements and add solvent if necessary.

   c. Touch the **Ok** button to begin column equilibration.

6. Prepare the test mix as follows:

   a. Add 4 mL of ethyl acetate to one of the vials and dissolve the sample by capping and shaking the vial (this may take a couple of minutes).

   b. Add 1 mL of hexanes (hexane, cyclohexane, heptane, or petroleum ether).

7. Select **Ok** to start the equilibration. After equilibration, the module will pause for sample injection. Draw all of the dissolved test sample then:

   a. Place the syringe on top of the Liquid Injection Accessory valve.

   b. Open the valve by turning the handle so it is in line with the tubing.

   c. Inject the liquid.

   d. Chase the sample with 10 mL of your A solvent to ensure the sample is clear of the injection port and valve.

   e. Close the valve by turning the handle so that it is perpendicular to the tubing. Remove the syringe.

   f. Touch the Ok button to continue the run.

8. When the system finishes the run, review the resulting 254 nm chromatogram which should meet the following criteria (refer to Figure 1):

   - Baseline must not rise more than 0.1 AU during separation.
   - Retention time of Peak 2 (time that the top of the second peak occurs) must be between 23.5 and 26.5 minutes.

If the criteria is not met, repeat the verification steps. If the results still do not meet the listed criteria, contact your Teledyne ISCO representative.

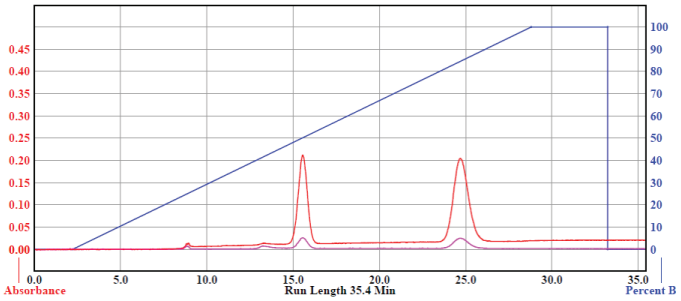

Figure 1: Universal Verification Chromatogram for silica gel

Section 2: Torrent AQ

Required Apparatus and Reagents

- Combi*Flash* Torrent Aq module with adapters installed for small column sizes.
- 275 gram RediSep® Gold C-18 column (69-2203-339)
- Water and methanol, minimum A.C.S. reagent grade (or equivalent)
- Universal Verification Kit (P/N 60-5234-317)

Purging

Before proceeding, the Combi*Flash* Torrent module should be ready to run with water (A solvent) and methanol (B solvent). If the system is using a different solvent system, or if this is the first run for a new system, the internal plumbing must be purged and reprimed.

Note

The Combi*Flash* Torrent module is shipped from the factory with isopropyl alcohol (IPA) in the internal solvent lines.

To purge the module:

1. Connect the solid load cartridge tubing to the Sample Injection Port.
2. Insert a column bypass tube.
3. Ensure that the Solvent A inlet line is receiving water, and Solvent B inlet lines are receiving methanol.
4. From the menu, select the **Tools>Manual** Control to open the **Manual Control** window.
5. Select the Through **Column & Cartridge Valve Position** setting.
6. Touch the **Prime B** button. Make sure the methanol is chosen as the B solvent, and the correct solvent line is in the methanol. The solvent pump will start.
7. After at least 400 mL of solvent has been pumped, touch the **Stop** button.
8. Touch the **Prime A** button. The solvent pump will start.
9. After at least 400 mL of solvent has been pumped, touch the Stop button.
10. Close the **Manual Control** window.
11. After the pressure gauge on the touch screen has returned to 0 psi, remove the column bypass tube and disconnect the solid load cartridge tubing from the Sample Injection Port.

Note

Some solvent may drain when removing the column bypass tube.

The Combi*Flash* Torrent module is now ready for the Verification Procedure.

Verification Procedure

1. Connect the Liquid Injection Accessory from the accessory kit to the Sample Injection Port.
2. Load the 275 gram column on the module. Rotate the column so the label faces the RFID antenna.
3. Create a gradient table with the following gradient points:

Solvents	Length (minutes)	%B
Methanol	Start	50
Methanol	12	100

- Set the flow rate to 100 mL/min
- Wavelength 1 set to 214 mn; wavelength 2 = 254 nm
- Set equilibration volume to 729 mL

4. Optionally, in the Peak Collection section of the display, select the **Peaks** option.
5. On the Main screen, touch the **Play** button. This opens the **Run Requirements** window.
   a. Select Liquid for the Sample Loading Technique.

   Select the Start Rack and **Start Tube** number for the fraction collector. If using the Fractionation Valve, the set of containers is considered a rack. The **Start Tube** number identifies which of the six valve positions to start from.

   b. Review the Solvent Requirements and add solvent if necessary.

   c. Touch the **Ok** button to begin column equilibration.
6. Prepare the test mix as follows:
   a. Add 4 mL of methanol to one of the vials and dissolve the sample by capping and shaking the vial (this may take a couple of minutes).

   b. Add 1 mL of water.

7. Select **Ok** to start the equilibration. After equilibration, the module will pause for sample injection. Draw all of the dissolved test sample then:

   a. Place the syringe on top of the Liquid Injection Accessory valve.

   b. Open the valve by turning the handle so it is in line with the tubing.

   c. Inject the liquid.

   d. Chase the sample with 10 mL of water to ensure the sample is clear of the injection port and valve.

   e. Close the valve by turning the handle so that it is perpendicular to the tubing. Remove the syringe.

   f. Touch the Ok button to continue the run.

8. When the system finishes the run, review the resulting 214 nm chromatogram which should meet the following criteria (refer to Figure 2):

   - Baseline must not rise more than 0.1 AU during separation.
   - Height of Peak 2 must be higher than Peak 1.
   - Retention time of Peak 2 (time that the top of the second peak occurs) must be between 7.5 and 9.5 minutes.

If the criteria is not met, repeat the verification steps. If the results still do not meet the listed criteria, contact your Teledyne ISCO representative.

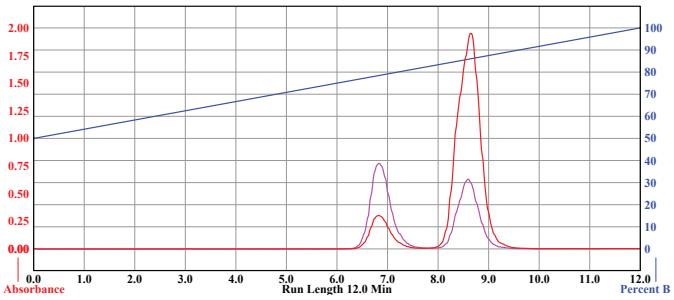

Figure 2: Universal Verification Chromatogram for 275 g C18 column with MeOH/Water.

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