

Model 340CF ELSD with CombiFlash Rf 200 System Verification

Using Test Mix C



Instruction Sheet P/N 60-5243-169
October 7, 2010

Background

This procedure is used to verify proper operation of Teledyne Isco's model 340CF ELSD when installed with a CombiFlash[®] Rf 200 system. The procedure can detect connection problems and errors in detector operation.

This system verification assumes:

- that you are familiar with the operation of the 340CF ELSD and CombiFlash Rf 200 system,
- The operation of the CombiFlash Rf 200 system has been verified previously using Test Sample NPHE (instruction sheet 69-3873-307),
- The 340CF ELSD module is installed according to the instruction manual (69-5243-095).

If concerns arise about operating either instrument, consult the user manual.

Required Apparatus and Reagents

- Model 340CF ELSD
- CombiFlash Rf 200 system
- 12 gram RediSep[®] silica gel column (69-2203-312)
- Hexane and ethyl acetate, minimum A.C.S. reagent grade (or equivalent)
- Test Mix C Kit (60-5247-017) or Test Mix C vial (60-5244-110).

Purging

Before proceeding, the CombiFlash Rf 200 system should be ready to run with ethyl acetate (B solvent) and hexane (A solvent). If the module is using a different solvent system, purge the internal solvent lines including the column solvent path, and then prime with ethyl acetate and hexane.

Verification Procedure

1. Ensure that the 340CF ELSD is connected to the CombiFlash Rf and ready to operate. Ensure that the following operating parameters are set:
 - SC (Spray Chamber) = 35 °C
 - DT (Drift Tube) = 45 °C
 - Filter = 5
 - GX = PREP
 - FS = 5V
 - CL = 20%
 - GAS = 65 ±5 psi
2. Prime the ELSD drain tube with ~10 mL of ethyl acetate.
3. Load the 12 gram column on the CombiFlash Rf.

4. Ensure the CombiFlash Rf automatically loads a default method for the column. If not, use the touch screen to manually select the RediSep column media and size.
5. Touch the Method Editor button to open the advanced settings.
6. In the Peak Collection section of the display, select the Peaks option.
7. In the Peak Detection section of the display:
 - a. Ensure the $\lambda 1$ option is enabled and the detector wavelength is 254 nm.
 - b. Touch the Details button for $\lambda 1$ and ensure that the Slope Based option is enabled with a 1 Min Peak Width setting, and the Threshold option is enabled with a 0.20 AU setting.
 - c. Touch OK to close the $\lambda 1$ details.
 - d. Enable the External Detector option.
 - e. Touch the Details button for the External Detector and ensure that the Slope Based option is enabled with a 1 Min Peak Width setting, and the Threshold option is enabled with a 0.20v setting.
 - f. Touch OK to close the External Detector details.
8. Touch the Exit button to close the Method Settings window and then select Yes to save the changes.
9. Touch the Play button to open the Run Requirements window.
 - a. Select Liquid for the Sample Loading Technique.
 - b. Select the Start Rack and Tube number for the fraction collector.
 - c. Review the Solvent Requirements and add solvent if necessary.
 - d. Touch the OK button to begin column equilibration.
10. Prepare the Test Mix C vial by completely dissolving the dry sample in 1 mL of ethyl acetate.

Note

The cholesterol will dissolve slowly. Sonication or vortexing may speed dissolution.

Note

The solution should be used within 24 hours or discarded.

11. After column equilibration, the CombiFlash Rf 200 system will pause for sample injection. Draw all of the dissolved Test Mix C into a syringe and inject it into the Sample Injection Port.

✓ Note

A chase solvent forces the sample through the automated injection valve.

12. Inject 0.25 mL of ethyl acetate as a chase solvent into the Sample Injection Port.
13. Touch the OK button to continue the run.

14. When the module finishes the run, review the resulting chromatogram which should meet the following criteria (refer to Figure 1):

- Peak 1 (cholesterol) should only appear on the External Detector trace.
- Peak 2 (4-aminobenzoic acid) should be visible on the External Detector and the 254 nm traces.
- Peak 2 on both traces should overlay within 0.5 minutes of each other.

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

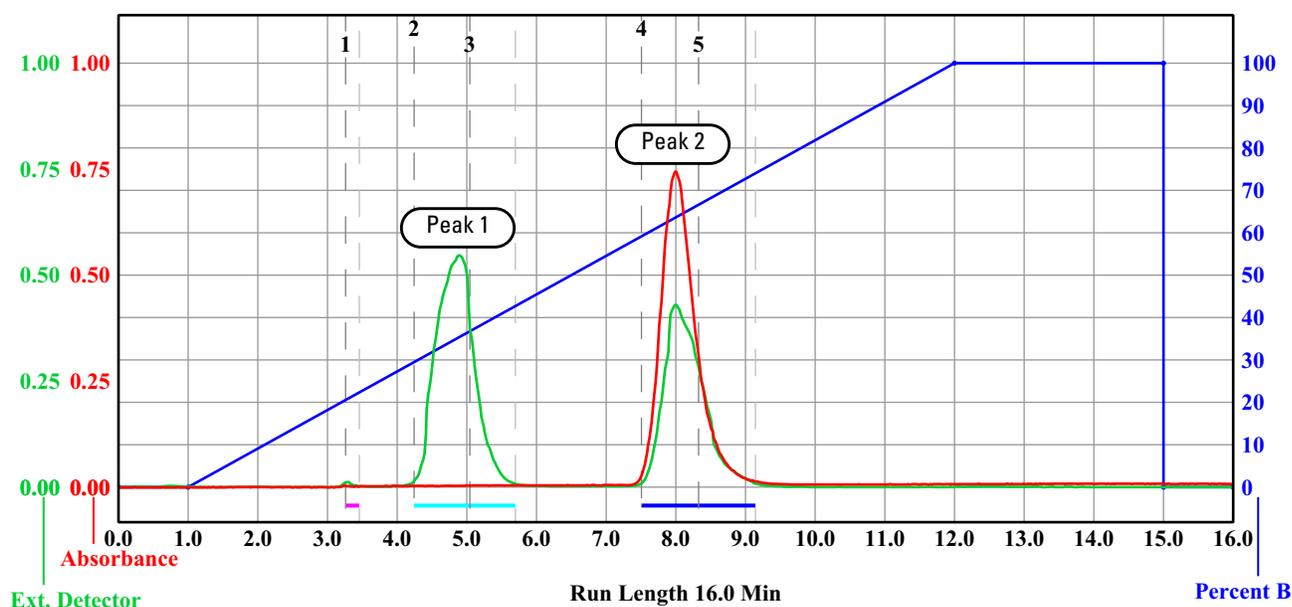


Figure 1: Test Mix C Chromatogram

Peak 1: cholesterol
Peak 2: 4-aminobenzoic acid

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