

CombiFlash[®] Rf System Verification

Using Test Sample NPHE



Application Note 21 | P/N 69-3873-244
Revision B, February 15, 2012

Background

This procedure is used to verify proper operation of Teledyne Isco's CombiFlash Rf System. 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 CombiFlash Rf system. If concerns arise about operating the system, consult the user manual.

Required Apparatus and Reagents

- CombiFlash Rf System equipped with Solid Load Cartridge Caps for cartridges *with locking tabs*. For cartridge caps that require sleeves, refer to instruction sheet 69-3873-307 (Application Note 11).
- One of the following CombiFlash Rf Test Kits:
 - Part number 60-5237-050 — this kit contains five of the 5 gram size solid load cartridges pre-filled with 0.5 grams of test sample NPHE, and five 4 gram RediSep Rf silica gel columns.
 - Part number 60-5237-051 — this kit contains four of the 25 gram size solid load cartridges pre-filled with 0.5 grams of test sample NPHE, and four 4 gram RediSep Rf silica gel columns.
- Ethyl acetate and hexane, minimum A.C.S. reagent grade (or equivalent)
- Waste reservoir

Procedure

1. Prime the Solvent B inlet line with ethyl acetate. Then, prime the Solvent A inlet with hexane.
2. Place a pre-loaded solid load cartridge from the test kit into the solid sample position.
3. Load a 4 gram silica gel column on the system.
4. For CombiFlash Rf systems with UV or UV-vis detection, use the default method for a 4 gram silica gel column (Table 1).

For CombiFlash Rf systems with evaporative light scattering detection (ELSD) detection, modify the default 4 gram silica gel column method to include ELS detection as shown in Table 2.

5. Run the separation.
6. Compare the chromatogram with Figure 1 (UV systems) or Figure 2 (ELSD systems). The figures include verification criteria.

If the chromatogram fails to meet the criteria, repeat steps 2 through 6. If the repeated separation fails to meet the criteria, contact your Teledyne Isco representative.

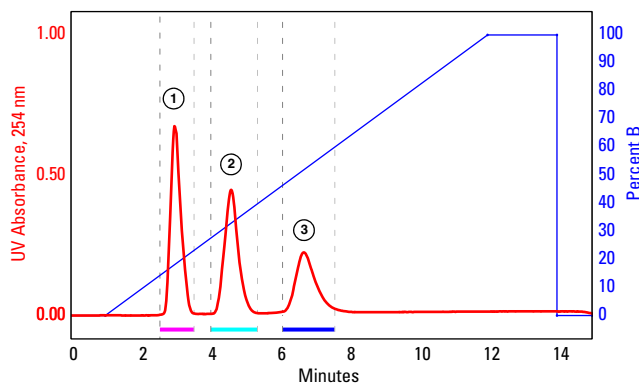
Table 1: Run Parameters for UV and UV-vis Systems

Wavelength:	254 nm	
Mobile phase:	Solvent A: hexane Solvent B: ethyl acetate	
Fraction Size:	18 mL	
Flow Rate:	18 mL/minute	
Equilibration Volume:	36 mL	
Gradient:	% B	Minutes
	0	Initial
	0	1
	100	11
	100	2
	0	0
	0	1

Table 2: Run Parameters for ELSD systems

Wavelength:	254 nm and ELS Detection – Spray Chamber Temp: 50 °C; Drift Tube Temp: 55 °C	
Mobile phase:	Solvent A: hexane Solvent B: ethyl acetate	
Fraction Size:	18 mL	
Flow Rate:	18 mL/minute	
Equilibration Volume:	36 mL	
Gradient:	% B	Minutes
	0	Initial
	0	1
	100	11
	100	2
	0	0
	0	1

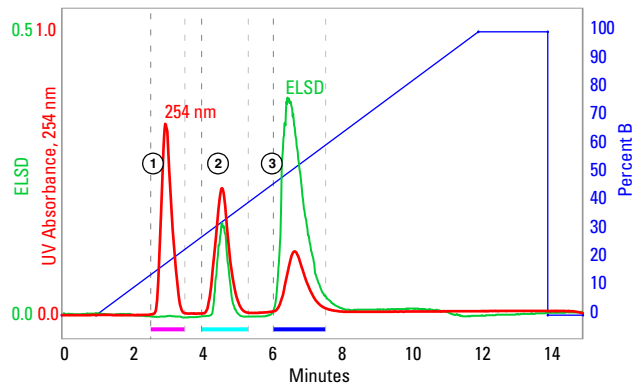
Peak 1: acetophenone
Peak 2: methylparaben
Peak 3: 4-aminobenzoic acid

**Figure 1: UV Reference Chromatogram**

Use the following criteria to evaluate the chromatogram:

1. Retention time of Peak 3 (time that the top of the third peak occurs) occurs at 7 ± 1 minute.
2. Baseline must not drift more than 0.1 AU during separation.
3. Height of Peak 2 should be higher than Peak 3.

Peak 1: acetophenone
Peak 2: methylparaben
Peak 3: 4-aminobenzoic acid

**Figure 2: UV and ELSD Reference Chromatogram**

Use the following criteria to evaluate the chromatogram:

UV Absorbance Trace (254 nm)

1. Retention time of Peak 3 (time that the top of the third peak occurs) occurs at 7 ± 1 minute.
2. Baseline must not drift more than 0.1 AU during separation.
3. Height of Peak 2 should be higher than Peak 3.

ELSD Detector Trace

4. Height of Peak 3 should be higher than Peak 2.
5. Peaks 2 and 3 of the ELSD and UV traces should align horizontally within ± 0.5 minute.
6. Peak 1 is generally not detected due to the volatility of acetophenone.

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