

CombiFlash[®] Rf System Verification

Using Test Sample NPHE



Chromatography Technical Note -TN24
P/N 69-3873-244 | Revision E, May 15, 2014

Background

This procedure is used to verify proper operation of the Teledyne Isco CombiFlash Rf system by detecting errors in solvent gradient formation. Proper alignment of the fraction collector is verified by the collection of peaks.

Note

These instructions do not apply to the CombiFlash Rf system paired with the Model 340CF ELSD. Systems with this external ELSD should be verified using Test Mix C and instruction sheet 60-5243-169 supplied with the Model 340CF ELSD.

Note

These instructions do not apply to the CombiFlash Rf⁺ Purlon mass-directed flash system. Purlon system operation should be verified using Test Mix MSD and Test Mix MSW supplied with the CombiFlash Rf⁺ Purlon system. Refer to Technical Note 25 for details.

This system verification assumes that the user is 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 appropriately sized Solid Load Cartridge Cap
- One of the following CombiFlash Rf Test Kits:
 - Part #60-5237-050 — contains five 5 g solid load cartridges pre-filled with 0.5 g of test sample NPHE, and five 4 g RediSep Rf silica gel columns
 - Part# 60-5237-051 — contains four 25 g solid load cartridges pre-filled with 0.5 g of test sample NPHE, and four 4 g RediSep Rf silica gel columns
- Ethyl acetate and hexane, minimum reagent A.C.S. grade (minimum 500 mL of each)
- Waste reservoir (minimum 1 L capacity)

Procedure

1. Prime the Solvent B inlet line with ethyl acetate.
2. Prime the Solvent A inlet line with hexane.
3. Place a pre-loaded solid load cartridge from the test kit into the solid sample position.
4. Load a 4 g RediSep Rf or RediSep Rf Gold[®] silica gel column on the system.
5. CombiFlash Rf Systems with UV or UV-vis Only.
Use the default method for a 4 g silica gel column as shown in Table 1.
CombiFlash Rf 200i Systems Only.
Modify the default method for a 4 g silica gel column to enable ELS detection as shown in Table 1.
6. Run the separation. When prompted to select a sample loading technique, select "Solid Load".
7. Compare the chromatogram with Figure 1. Table 2 includes verification criteria for peak elution times.

If the chromatogram fails to meet the verification criteria, repeat steps 3-7. If the repeated separation fails to meet the criteria, contact your Teledyne Isco representative.

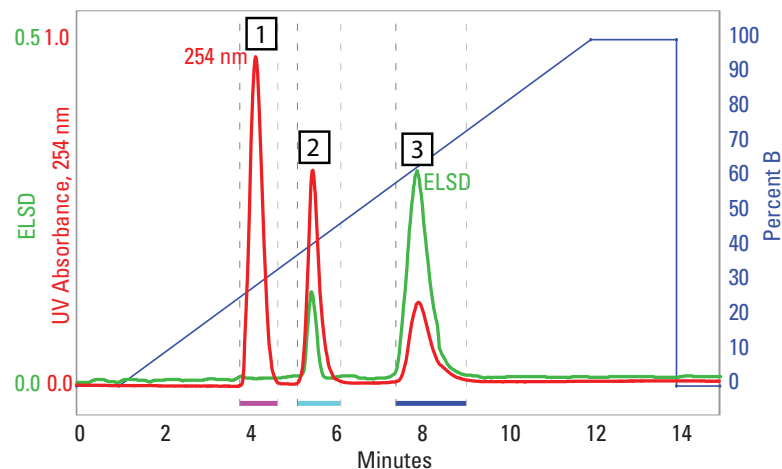


Figure 1: UV and ELSD Reference Chromatogram

Ensure the following criteria is met:

UV Absorbance Trace (254 nm)

- Baseline must not drift more than 0.1 AU during separation
- Height of Peak 2 should be greater than Peak 3

ELSD Detector Trace

- Peak 3 of the ELSD and UV traces should align horizontally within ± 0.5 minute
- Due to the volatility of the compounds under the recommended operating conditions, Peak 1 is generally never detected and Peak 2 may or may not be detected

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

Wavelength	254 nm	
Mobile Phase	Solvent A: hexane Solvent B: ethyl acetate	
Flow Rate	18 mL/minute	
Equilibration Volume	36 mL	
Gradient	%B	Minutes
	0	Initial
	0	1
	100	11
	100	2
	0	0
	0	1
<u>Systems with ELSD Only.</u> Verify the temperatures are set to the default normal phase values:		
Spray Chamber Temperature	30° C	
Drift Tube Temperature	60° C	

Table 2: Peak Elution Times

System	Peak #	Acceptable Retention (Minutes)	Acceptable Deviation (Minutes)
Rf150	2	7.8	± 1.0
Rf200	2	5.0	± 1.0
Rf200i	3	8.0	± 1.0
Rf+	2	5.7	± 1.0
Rf+ Lumen	3	9.0	± 1.0

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