

CombiFlash[®] System Verification

Using Test Sample A

Background

This procedure is used to verify proper operation of Teledyne Isco CombiFlash Systems. It can detect errors in solvent gradient formation, detection sensitivity, and detection wavelength. Proper operation of the fraction collector is evidenced by the collection of peaks.

These procedures assume that you are familiar with the operation of the CombiFlash System. If concerns arise about functioning of the system, consult the instrument's user manual.

Required Apparatus and Reagents

- CombiFlash System
- Pre-packaged CombiFlash Test Kit, part number 68-3877-010. The kit includes five of the 5g size solid load cartridges pre-filled with 0.5g of Test Sample A, and five of 4g normal phase RediSep[®] columns.
- Part number 60-3877-053 — this kit contains four of the 25 gram size solid load cartridges pre-filled with 0.5 grams of test sample A, and four of the 4 gram RediSep Rf silica gel columns.
- Ethyl acetate and hexane, minimum A.C.S. reagent grade (or equivalent).

Note

CombiFlash 4x Systems Only: Before proceeding, configure the Companion[®] for single channel operation. Disconnect four interconnecting solvent lines from Companion's liquid ports A through D. Insert factory U-shaped stainless steel tubes (stored in the drawer at the base of the 4x module) between ports A-B and C-D. Restore solvent lines after performing Test Sample A procedures.

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. Place a 4g normal phase RediSep column in the corresponding position.
4. Using the parameters in Table 1, run one separation to verify that system is functioning properly.

Verification

Figure 1 is an example of a normal Test Sample A separation. Compare the resulting chromatogram with this figure. If the chromatogram meets the listed criteria, it is not necessary to repeat the procedure.

Table 1: Run Parameters for Test Sample A Separation^a

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:	% Solvent B	Minutes
	0	Initial
	0	1
	100	11
	100	2
	0	0
	0	1

- a. To verify CombiFlash OptiX[™] 10 systems, enter the parameters using the Experiment and Gradient Editor windows. To verify CombiFlash Companion and Sq 16x systems, enter the run parameters or use Teledyne Isco's 4g Default Method.

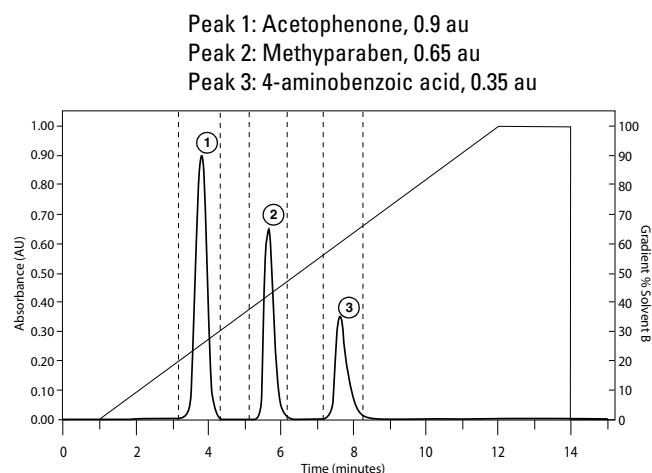


Figure 1: Normal Chromatogram Use the following criteria to evaluate the chromatogram:

1. Retention time of Peak 3 (time that the top of the third peak occurs) occurs at 7.5 ± 0.25 minutes.
2. Baseline must not drift more than 0.1 AU during separation.
3. Height of Peak 2 should be 1.5 to 2 times higher than height of Peak 3.

Troubleshooting

If the chromatogram fails to meet the criteria, refer to Figures 2 through 5 for possible causes.

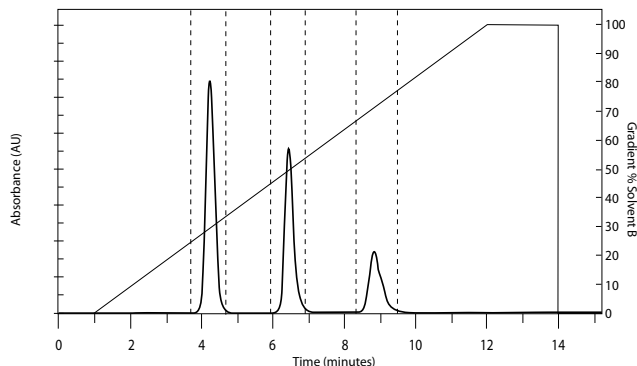


Figure 2: Low percentage of solvent B Low solvent %B mixing retains peak 3 longer than necessary.

Errors in retention time may be caused by flow rate errors or gradient composition errors. Baseline drift upwards could be due to actual wavelength being lower than programmed wavelength. If peak height of Peak 2 is not greater than Peak 3, the wavelength is likely to be too high.

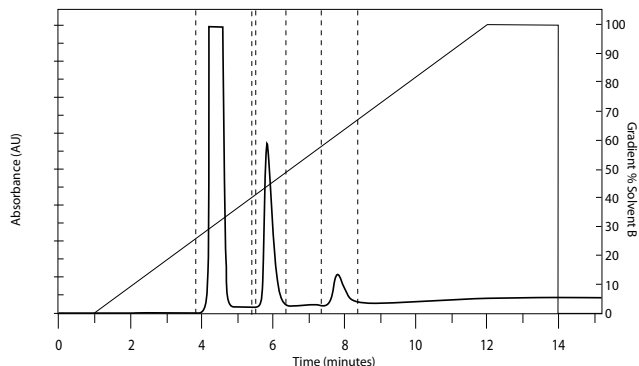


Figure 4: Wavelength too low Low wavelength causes a baseline drift as %B solvent interferes with absorbance.

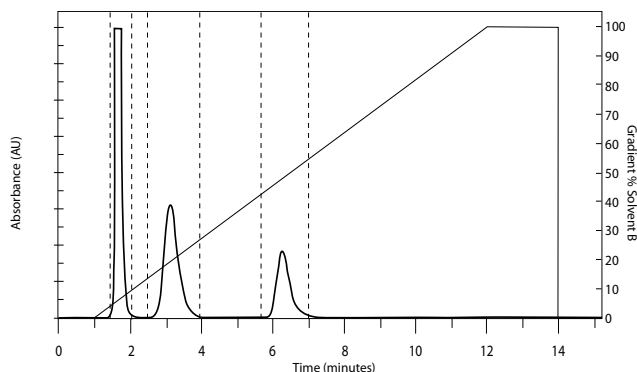


Figure 3: High percentage of solvent B High solvent %B mixing causes the peaks to elute out quicker.

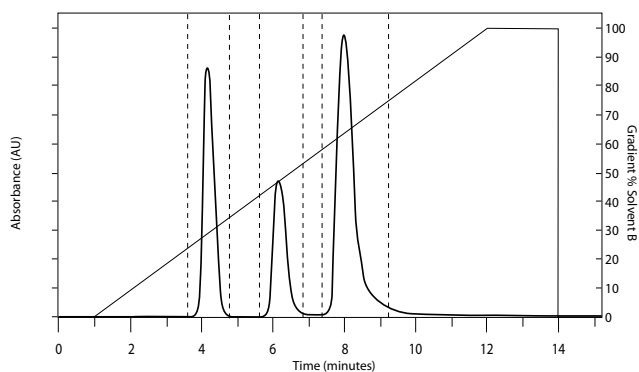


Figure 5: Wavelength too high A high wavelength causes Peak 3 to become greater in size than Peak 2.

Note

The retention time is dependent upon the degree of %B mixing.

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