

CombiFlash[®] Rf System Verification

Using Test Sample NPHE

Chromatography Application Note 21
P/N 69-3873-244
Revision A, June 17, 2010

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. Using the parameters in Table 1 or the default method for a 4 gram silica gel column, run one separation.

Verification

Figure 1 is an example of a normal test sample NPHE separation. Compare the resulting chromatogram with

this figure. If the obtained chromatogram is comparable to Figure 1, it is not necessary to repeat the procedure.

Table 1: Run Parameters for Test Sample NPHE Separation

| | |
|-----------------------|---|
| 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: | % BMinutes 0Initial 01 10011 1002 00 01 |

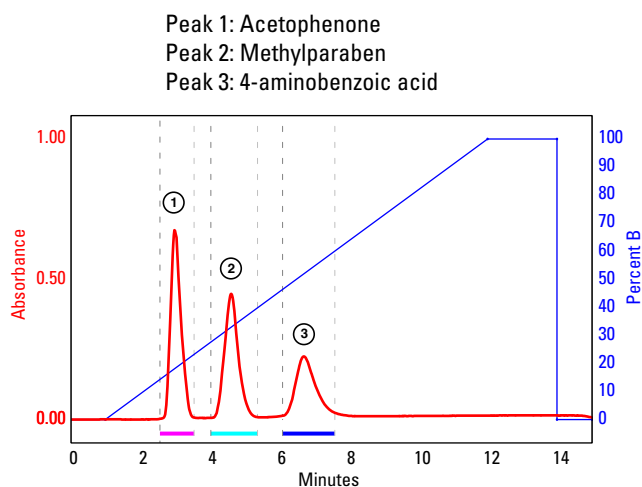


Figure 1: NPHE Chromatogram Use the following criteria to evaluate the chromatogram:

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.

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