

CombiFlash[®] Rf⁺ Purlon System Verification

Using Test Samples MSW and MSD

Background

This procedure is used to verify proper operation of the Teledyne Isco CombiFlash Rf⁺ Purlon Flash chromatography 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 system. CombiFlash Rf system operation should be verified using Test Sample NPHE. Refer to Technical Note 24 for details.

This system verification assumes that the user is familiar with the operation of the CombiFlash Rf⁺ Purlon system. If concerns arise about operating the system, consult the user manual.

Required Apparatus and Reagents

- CombiFlash Rf⁺ Purlon system equipped with appropriately sized Solid Load Cartridge Cap and the either of the following:
 - Part #69-3873-235 — contains (30) 5 g empty sample load cartridges with lock
 - Part #69-3873-240 — contains (30) 25 g empty sample load cartridges with lock
- Test Mix MSD (Part #60-5234-626) provided in the MS Accessory Kit
- Test Mix MSW (Part #60-5234-627) provided in the MS Accessory Kit
- Ethyl acetate and hexane, minimum reagent A.C.S. grade (minimum 1 L of each)
- Methanol with 0.1% formic acid, LC-MS grade (minimum 100 mL) for use as carrier solvent
- Waste reservoir (minimum 2 L capacity)

Procedure Using MSW Test Mix

1. Verify the Purlon is fitted with the ESI probe.
2. Place the carrier fluid pump inlet line into the reservoir containing the methanol/0.1% formic acid solution.

3. Prime the carrier fluid pump on the fluid interface using the 5 mL syringe provided.
4. From the MS menu, verify the Purlon is in “Operate” mode. If it is not, select “Operate” from the MS menu and allow five minutes for the heaters to warm up.
5. Select “Method Development” from the MS menu.
6. Set the Start Mass at 200 and the End Mass at 600.
7. Verify that the Polarity is set to “Positive”.
8. Verify the Ion Setting is set to “TYPICAL”.
9. Using the 1 mL syringe and needle supplied in the MS Accessory Kit, withdraw 100 µL of MSW solution.
10. Switch the manual injection valve of the Purlon to the “Load” position. Insert the 1 mL syringe filled with sample in the injection port and depress the syringe plunger to load the 100 µL sample into the sample loop. Switch the manual injection valve to the “Scan Mass” position.
11. Compare the mass spectrum with Figure 1. Table 1 includes the expected [M+1] values. If the displayed spectrum fails to display the same values, repeat steps 2-10.
If the repeated results fail to match the values, contact your Teledyne Isco representative.

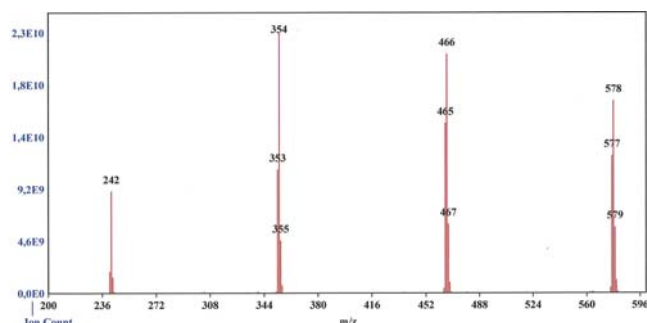


Figure 1: Expected results using MSW Test Mix

Table 1: Compounds in MSW Test Mix

Test Compounds	Molecular Weight	M+1 (minus loss of Bromine 79)
tetrabutylammonium bromide	321	242
tetrahexylammonium bromide	433	354
tetraoctylammonium bromide	545	466
tetradecylammonium bromide	657	578

Note

Run the MSD test mix only after running and passing the MSW test mix.

Procedure Using MSD Test Mix

1. Prime the Solvent B inlet line with ethyl acetate.
2. Prime the Solvent A inlet line with hexane.
3. Select an empty solid load cartridge and weigh out 500 mg of MSD test mix (Part #60-5234-626). If a balance is unavailable, when using a 25 g solid load cartridge, load the MSD sample to a depth of 2 mm. If using a 5 g solid load cartridge, load the MSD sample to a depth of 4 mm. Insert the top frit into the cartridge and press into position using the guide rod.
4. Place the prepared solid load cartridge into the solid sample position.
5. Load a 12 g RediSep Rf or RediSep Rf Gold[®] silica gel column on the system.
6. Use the default conditions for the 12 g RediSep Rf column. If using the RediSep Rf Gold[®] column, select “Gold Resolution” when prompted. Refer to Table 2 for run parameters.
7. Press the Play button. Set the Target Masses to 180 and 185.
8. If the system is fitted with an integrated ELSD, verify that it is activated.
9. Run the separation. When prompted to select a sample loading technique, select “Solid Load” and click “OK”.
10. Compare the chromatogram generated with Figure 2. Table 3 includes compounds and expected retention times for the MSD test mix.

If the chromatogram fails to meet the verification criteria, repeat steps 4-8. If the repeated separation fails to meet the criteria, contact your Teledyne Isco representative.

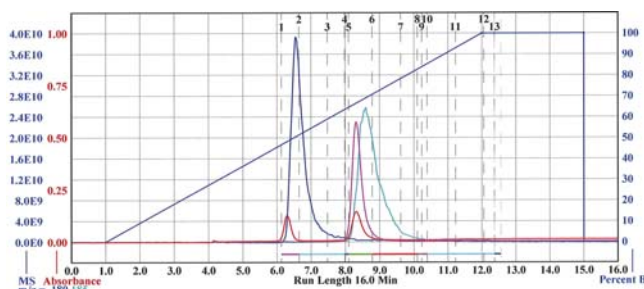


Figure 2: Expected results using MSD Test Mix

Table 2: Run Parameters for CombiFlash Rf Systems

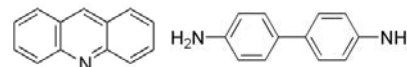
Wavelength	254 nm and 280 nm	
Mobile Phase	Solvent A: hexane Solvent B: ethyl acetate	
Flow Rate	30 mL/minute	
Equilibration Volume	101 mL	
Gradient	%B	Minutes
	0	Initial
	0	1
	100	11
	100	3
	0	0
	0	1

Systems with ELSD Only. Verify the temperatures are set to the default normal phase values:

Spray Chamber Temperature	30° C
Drift Tube Temperature	60° C

Table 3: Compounds and Retention Times for MSD Test Mix using RediSep Rf Gold[®] Columns*

Compound Name	Peak 1	Peak 2
	Acridine	Benzidine
MW	179	184
[M+1] ⁺	180	185
[M-NH ₂] ⁺	N/A	168
RT Rf200	5.0 ± 1.0/minute	8.2 ± 1.0/minute
RT Rf200i	5.5 ± 1.0/minute	8.2 ± 1.0/minute
RT Rf ⁺	6.2 ± 1.0/minute	8.6 ± 1.0/minute
RT Rf ⁺ Lumen	6.7 ± 1.0/minute	8.6 ± 1.0/minute
UV at 254 nm	Detected	Detected
UV at 280 nm	Not Detected	Detected



*If using RediSep Rf columns, Peak 1 elutes 0.3 minutes earlier than the corresponding peak when using RediSep Rf Gold[®] columns. Peak 2 elutes one minute later.

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