CombiFlash® System Verification Instructions

Using Universal Verification Kit (60-5234-317)

TELEDYNE ISCO
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Instruction Sheet #69-5233-870
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Overview

These instructions are applicable to the CombiFlash systems, including: NextGen 100, NextGen 300, and NextGen 300+, Rf+, Rf+ Lumen, and Rf+ PurIon. This document describes the use of the Universal Verification Kit to verify the operation of the CombiFlash systems. The kit verifies operation of systems fitted with UV/UV-vis, ELSD, or MS detectors.

Universal Verification Kit Description

Each vial contains 50 mg of Phenacetin and 200 mg N-Benzylbenzamide. The system may be verified with either normal or reverse phase solvent systems.

Normal Phase Operational Verification-Liquid Load

Each vial contains 50 mg of Phenacetin and 200 mg N-Benzylbenzamide. The system may be verified with either normal or reverse phase solvent systems.

Normal Phase Operational Verification–Liquid Load

Follow the system priming directions as described in the CombiFlash NextGen Installation and Operation Guide (available as a PDF download from www.teledyneisco.com) Section 2.2.4, or in Section 2.1.4 of the CombiFlash Rf+ User Manual for Rf+ models. After priming the system, follow the steps below to verify operation:

1. Install one of the 12 gram RediSep Rf Gold® silica columns provided in the kit and select GOLD RESOLUTION when prompted. Use the pre-defined method.
2. Add 4 mL of ethyl acetate to one of the vials and dissolve the sample by capping and shaking the vial (this may take a couple of minutes).
3. Add 1 mL of hexanes (hexane, cyclohexane, heptane, or petroleum ether).
4. Use the default gradient and flow rate for the installed column.
5. If an ELSD is installed, use the factory default spray chamber and drift tube temperature settings.
6. Verify that the CombiFlash NextGen with ELSD is set to the default conditions (Sensitivity = NORMAL, Gain = 2). If the unit is a CombiFlash Rf+ Lumen, verify that the default conditions are being used (Gain = 1).
7. If a PurIon is installed, use the TYPICAL ion settings. Set PurIon loading to LOW on the RUN REQUIREMENT screen. Use masses of 180 and 212 Da, positive ionization. Carrier solvent should be either methanol or acetonitrile with 0.1% acid (formic or TFA). Conditions are valid whether running ESI or APCI interface.
8. Set the UV detector to 254, 280 nm. Perform a LIQUID LOAD and inject 1.0 mL of the sample mixture prepared in steps 2 and 3 directly onto the column.

Expected Results for NextGen Models:

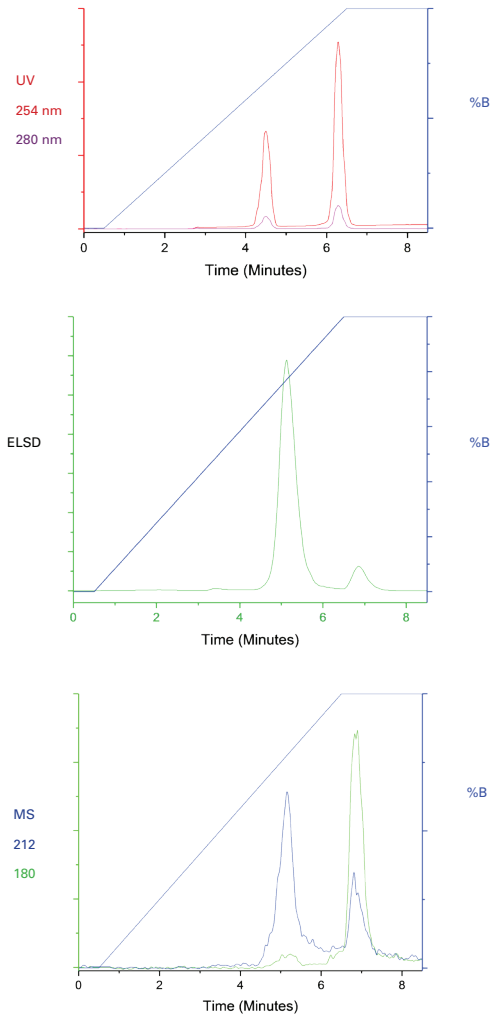

Figure 1: Normal Phase Detector Response
Please refer to the table for exact retentions times as different configurations have different retention times.

Expected Retention Time- Liquid Load		
NextGen, 100, 300, and 300+	Peak 1 (± 0.5) Minutes	Peak 2 (± 0.5) Minutes
UV/ UV-vis only	4.5	6.3
With PurIon	4.8	6.7
With ELSD	5.0	6.7
With ELSD and PurIon	5.1	6.8
CombiFlash Rf+	6.5	9.0
CombiFlash Rf+ Lumen	7.0	9.5

Normal Phase Operational Verification–Solid Load

As an alternative loading technique, the verification sample can be injected using a sample load cartridge and associated solid load cartridge cap.

Follow the system priming directions as described in the CombiFlash NextGen Installation and Operation Guide (available as a PDF download from www.teledyneisco.com) Section 2.2.4, or in Section 2.1.4 of the CombiFlash Rf+ User Manual for Rf+ models. After priming the system, follow the steps below to verify operation:

1. Choose between a 5 gram solid load cartridge packed with 2.5 grams of silica (P/N 69-3873-238) or a 25 gram cartridge packed with 12 grams of silica (P/N 68-3873-243).
2. Add 4 mL of ethyl acetate to one of the vials and dissolve the sample by capping and shaking the vial (this may take a couple of minutes).
3. Add 1 mL of hexanes (hexane, cyclohexane, heptane, or petroleum ether).
4. Inject 1 mL of the resulting sample onto the surface of the selected cartridge.
5. Place the cartridge onto the corresponding Solid Load Cartridge Cap (SLCC) and install into the system.
6. Install one of the 12 gram RediSep Rf Gold® silica columns provided in the kit and select GOLD RESOLUTION when prompted. Use the pre-defined method.
7. Use the default gradient and flow rate for the installed column.
8. If an ELSD is installed, use the factory default spray chamber and drift tube temperature settings.
9. Verify that the CombiFlash NextGen with ELSD is set to the default conditions (Sensitivity = NORMAL, Gain = 2). If the unit is a CombiFlash Rf+ Lumen, verify that the default conditions are being used (Gain = 1).
10. If a PurIon is installed, use the TYPICAL ion settings. Set PurIon loading to LOW on the RUN REQUIREMENT screen. Use masses of 180 and 212 Da, positive ionization. Carrier solvent should be either methanol or acetonitrile with 0.1% acid (formic or TFA). Conditions are valid whether running ESI or APCI interface.
11. Set the UV detector to 254, 280 nm.
12. Select PLAY and then select SOLID LOAD as the injection option.

Expected Retention Time–Solid Load		
Using 5 gram Solid Load Cartridge		
NextGen, 100, 300, and 300+	Peak 1 (± 0.5) Minutes	Peak 2 (± 0.5) Minutes
UV/ UV-vis only	4.6	6.5
With PurIon	4.9	6.9
With ELSD	5.1	6.9
With ELSD and PurIon	5.2	7.0
Expected RetentionTime–Solid Load		
Using 25 gram Solid Load cartridge		
NextGen, 100, 300, and 300+	Peak 1 (± 0.5) Minutes	Peak 2 (± 0.5) Minutes
UV/UV-vis only	5.9	8.1
With PurIon	6.1	8.7
With ELSD	6.1	8.7
With ELSD and PurIon	6.5	8.8

Reverse Phase Operational Verification

Follow the system priming directions as described in the CombiFlash NextGen Installation and Operation Guide (available as a PDF download from www.teledyneisco.com) Section 2.2.4, or in Section 2.1.4 of the CombiFlash Rf+ User Manual for Rf+ models. After priming the system, follow the steps below to verify operation:

1. Install a 15.5 gram RediSep Rf Gold C18 column (P/N 69-2203-334). This column needs to be purchased separately. Use the default method.
2. Add 4 mL of methanol or acetonitrile to one of the vials and dissolve the sample by capping and shaking the vial (this may take a couple of minutes).
3. Add 1 mL of water.
4. Use the default gradient and flow rate for the installed column.
5. If an ELSD is installed, use the factory default spray chamber and drift tube temperature settings.
6. Verify that the CombiFlash NextGen with ELSD is set to the default conditions (Sensitivity = SENSITIVE, Gain = 2). If the unit is a CombiFlash Rf+ Lumen, verify that the default conditions are being used (Gain = 1).
7. If a PurIon is installed, use the TYPICAL ion settings. Set PurIon loading to LOW on the RUN REQUIREMENT screen. Use masses of 180 and 212 Da, positive ionization. Carrier solvent should be either methanol or acetonitrile with 0.1% acid (formic or TFA). Conditions are valid whether running ESI or APCI interface.
8. Set the UV detector to 214, 254 nm.
9. Perform a LIQUID LOAD and inject 1.0 mL of the sample mixture prepared in steps 2 and 3 directly onto the column.

Expected Results for NextGen Models:

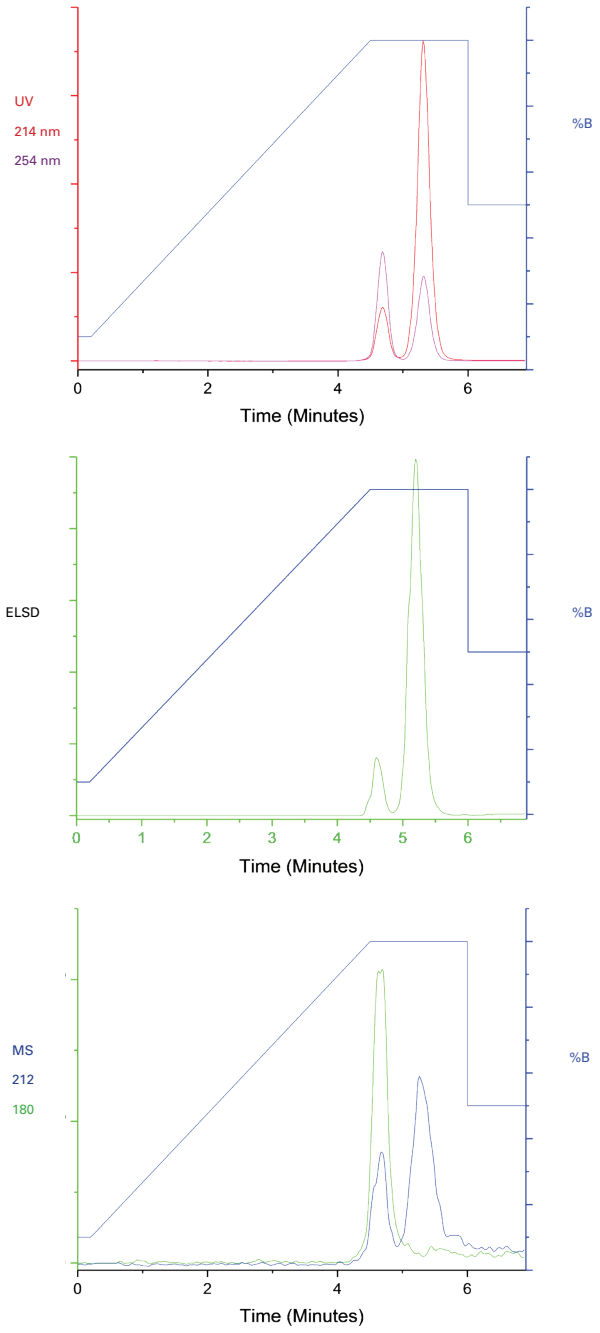

Figure 2: Reverse Phase Detector Response
Please refer to the table for exact retentions times as different configurations have different retention times.

Expected Retention Time in Minutes		
NextGen, 100, 300, and 300+	Peak 1 (± 0.5) Minutes	Peak 2 (± 0.5) Minutes
UV/ UV-vis only	4.5	5.1
With PurIon	4.7	5.3
With ELSD	4.6	5.2
With ELSD and PurIon	4.8	5.3

Troubleshooting

Problem	Causes and Resolutions
Peaks elute at or near void.	This is caused by strong solvent causing the peaks to elute too early. Causes include: -Solvent lines reversed. -Solvent line(s) in wrong solvent. -Wrong solvent(s) chosen for run. -Contaminated solvent bottles.
Peaks have poor shape.	Sample exposed to strong solvent during run. -Sample dissolved incorrectly (100% strong solvent, usually affects first peak more). -Test sample dissolved for C18 run on silica. -Test sample dissolved for silica run on C18. -Strong solvent in system–a second run should run fine in this case. -Leak- loose solvent line.
Sample fails to elute or elutes late.	Strong solvent isn't properly running through the column. -"B" solvent line not in solvent bottle. -Wrong solvent line chosen for B solvent (no solvent or a weak solvent being delivered). -Leak (loose "B" solvent line). -Contaminated "B" solvent.
Peaks elute early with little or no resolution.	Possible weak "A" solvent delivery problem. -"A" solvent line loose. -Wrong solvent line chosen. -Contaminated "B" solvent.
Weak or no UV signal for sample.	There are two causes for this: 1. Sample didn't elute. 2. Flow Cell contamination. -Flush flow cell with 2-propanol, absolute ethanol, or acetone followed by "B" solvent, followed by "A" solvent. -Verify sample volume injected.
Weak ELSD signal.	-Fill "P" Trap. -Verify correct settings used.
Weak mass spectrometer signal.	-Verify nitrogen pressure to mass spectrometer. -Verify ion settings. -Verify tune files loaded. -Confirm carrier solvent bottle is filled. Prime carrier solvent. -Verify carrier solvent prime valve is closed.
Mass spectrometer signal doesn't align with UV peaks.	-Verify carrier solvent prime valve closed. -Verify correct delay tubing installation. -Verify correct delay tubing value in system configuration.
Split peaks.	-Poor injection. -Column problem.

Should you need assistance with these instructions, please contact Teledyne ISCO.

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