EZ Prep System Verification

Using Universal Verification Kit (60-5234-317)

TELEDYNE ISCO
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Instruction Sheet #69-5233-949
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Overview

This document describes the use of the Universal Verification Kit to verify the operation of the EZ Prep systems. The kit verifies operation of system 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

Follow the system priming directions as described in the EZ Prep Installation and Operation Guide (available as a PDF download from www.teledyneisco.com). 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.
   - Verify that the ELSD is set to the default conditions (Sensitivity = NORMAL, Gain = 2).
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 EZ Prep:

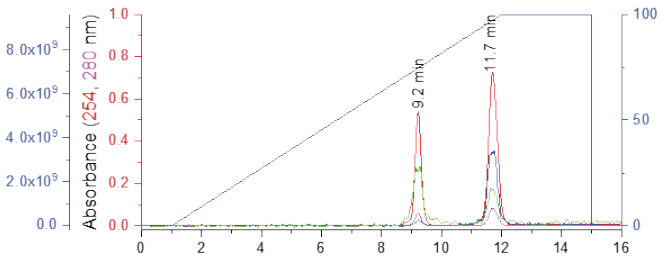

Figure 1: Normal Phase Detector Response

Expected RetentionTime- Liquid Load		
EZ Prep	Peak 1 (± 1.0) Minutes	Peak 2 (± 1.0) Minutes
UV/ UV-vis only	7.2	9.8
With PurIon	9.2	11.7
With ELSD	9.0	11.5
With ELSD and PurIon	9.2	11.7

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.

After priming the system, follow the steps below to verify operation:

1. Choose between a 5 grams 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 grams 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.
   - Verify that the ELSD is set to the default conditions (Sensitivity = NORMAL, Gain = 2).
9. 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.
10. Set the UV detector to 254, 280 nm.
11. Select PLAY and then select SOLID LOAD as the injection option.

Expected RetentionTime–Solid Load		
Using 5 gram solid load cartridge		
EZ Prep	Peak 1 (± 1) Minutes	Peak 2 (± 1) Minutes
UV/ UV-vis only	7.3	9.9
With PurIon	9.3	11.8
With ELSD	9.1	11.6
With ELSD and PurIon	9.3	11.8
Expected RetentionTime–Solid Load		
Using 25 gram Solid Load cartridge		
EZ Prep	Peak 1 (± 1) Minutes	Peak 2 (± 1) Minutes
UV/UV-vis only	8.6	11.6
With PurIon	10.5	13.7
With ELSD	10.3	13.5
With ELSD and PurIon	10.5	13.7

Prep HPLC Operational Verification

The Prep HPLC reverse phase verification method assumes the use of a 5 mL sample loop with a RediSep Prep C18 20 x 150 mm column which has been stored in 50:50 methanol:water mixture. Use of other sample loop sizes, columns, or storage mixtures may have an impact on retention times.

Follow the system priming directions as described in the EZ Prep Installation and Operation Guide (available as a PDF download from www.teledyneisco.com). After priming the system, follow the steps below to verify operation:

1. Install a ResiSep Prep C18 20 x 150 mm or equivalent column.
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. Open the method editor.
   - Set Equilibration Volume: 90 mL
   - Set Flow Rate: 18.9 mL/min
   - Set Fraction Collection: Peaks only
5. If an ELSD is installed, use the factory default spray chamber and drift tube temperature settings.
   - Verify that the ELSD is set to the default conditions (Sensitivity = SENSITIVE, Gain = 2).
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. Choose methanol as the B solvent.
10. Press PLAY.
11. Select the injection method appropriate for the system. If using AutoSampler or AutoInjector choose 1 injection of 1.0 mL. If performing a manual injection:
    a. Choose LOAD AFTER EQUILIBRATION.
    b. Start equilibration.
    c. After equilibration, and when prompted, inject 1.0 mL of Verification Mix and leave the syringe in place until the injection valve moves. Once the valve rotates, flush the port with 1 mL of methanol or acetonitrile.

Expected Results for EZ Prep:

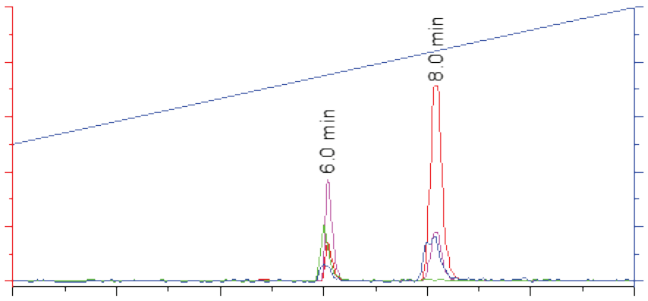

Figure 2: Prep HPLC Reverse Phase Detector Response

Expected RetentionTime—Prep HPLC Reverse Phase		
EZ Prep	Peak 1 (± 0.5) Minutes	Peak 2 (± 0.5) Minutes
UV/ UV-vis only	5.3	7.3
With PurIon	6.0	8.0
With ELSD	5.8	7.8
With ELSD and PurIon	6.0	8.0

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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