

ACCQPrep Verification Instructions

Using ACCQPrep Verification Kit (60-5234-835)

Overview

This document describes the use of the Universal Verification Kit to verify operation of the ACCQPrep HP125/150 system and expected results along with troubleshooting information for potential problems.

Universal Verification Kit Description

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

Reverse Phase Operational Verification

The verification method assumes the use of a 5 mL sample loop with a RediSep® Prep C18 20 x150mm column which has been stored in a 50/50 methanol: water mixture. Use of other sample loop sizes, columns, or storage mixtures may have an impact on retention times.

1. 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).
2. Add 1 mL of water.
3. Install a RediSep Prep C18 20 x 150 mm or equivalent column.
4. Set Equilibration volume: 90 mL
5. Set Flow Rate: 18.9 mL/min
6. Set Fraction Collection: Peaks only
7. Program gradient conditions
 - a. If an ELSD is installed, use the factory default spray chamber and drift tube temperature settings.

Duration (Minutes)	%B
0	50
12	100

8. Verify that the ACCQPrep with ELSD is set to the default conditions (Sensitivity = HIGH, GAIN = 1); Spray chamber: 20 °C; Drift tube 60 °C.
9. If a Purlon is installed, use the TYPICAL ion settings. Set Purlon loading to LOW on the RUN REQUIREMENTS 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 214, 254.
11. Choose your appropriate solvents. Either methanol or acetonitrile can be used as the Bsolvent.
12. Press PLAY.
13. Select the injection method appropriate for the system. If performing a manual injection:
 - a. Choose LOAD AFTER EQUILIBRATION.
 - b. Start equilibration.
 - c. After equilibration, and when prompted, inject 0.2 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 Retention Time - Methanol		
ACCQPrep HP125/150	Peak 1 (± 0.5) Minutes	Peak 2 (± 0.5) Minutes
UV/ UV-Vis only	4.6	6.4
With Purlon	4.6	6.5
With ELSD	4.9	6.8
With ELSD and Purlon	4.9	6.9
Expected Retention Time - Acetonitrile		
UV/ UV-Vis only	2.9	4.5
With Purlon	2.9	4.5
With ELSD	3.2	4.9
With ELSD and Purlon	3.2	4.9
Expected UV Absorbance at 214 nm		
UV	Peak 1 > Peak 2	

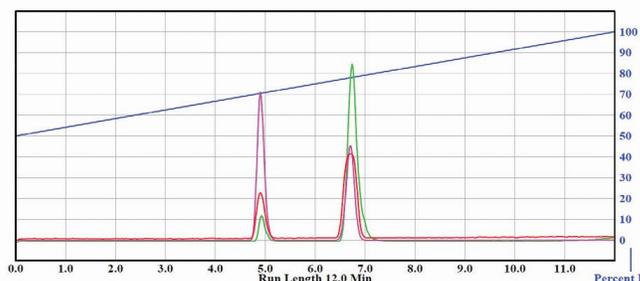


Figure 1: Water:Methanol Result

Troubleshooting

<p>Peaks do not match the graph.</p>	<p>Peak heights can vary by up to 50% due to small variances in peak width, solvents left in column before separation, loop flushing technique (manual injection only), etc.</p> <p>Retention time may vary due to the presence of modifiers such as TFA, differences between columns, unknown solvents remaining in column before separation started, insufficient injection loop flushing (manual injection only), etc.</p>
<p>Peaks elute too early.</p>	<p>Using a column other than a RediSep Prep C18 20 x 150 mm.</p> <p>The typical results shown assume 50:50 water:methanol is in column before starting this procedure. Stronger solvents in the column may impact the retention time.</p> <p>Residual solvents left in the ACCQ.Prep can affect retention time. If unknown fluids are in the system, flush the lines with isopropyl alcohol before performing PRIME with the proper solvents.</p> <p>Loop not flushed before injection (manual injection only).</p>
<p>Peaks elute too late.</p>	<p>Using a column other than a RediSep Prep C18 20 x 150 mm.</p> <p>Check flow rate (collect fractions and inspect for correct volume). May need to reprime the pumps due to air in pump heads.</p> <p>Using a 20x250 mm column will cause peak to elute ~67% later and will have a wider retention time variation.</p> <p>An ACCQ.Prep installed with an ELSD or MS may have slightly delayed retention times due to additional plumbing. Recheck correct installation of tubing to MS.</p> <p>Residual solvents left in the ACCQ.Prep can affect retention time. If unknown fluids are in the system, flush the lines with isopropyl alcohol before performing priming with the proper solvents. Peak 1 wider than peak 2. Injection loop not properly flushed (manual injection only).</p>

<p>No peaks or peaks very small.</p>	<p>Sample injected before the play button is pressed and within 1 minute of previous separation (manual injection only). Rotor in injection valve assembled incorrectly.</p>
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