ACCQ*Prep* Verification Instructions

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Instruction Sheet 60-5233-811
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Overview

This document describes the preparation of the ACCQ*Prep* Verification mixture, the verification method, and expected results along with troubleshooting information for potential problems.

Verification Mix Description and Preparation

The ACCQ*Prep* verification mixture is 1 mg/mL each of methyl and propyl paraben (methyl and propyl 4-hydroxybenzoate) dissolved in 50:50 water:methanol. Acetonitrile can be used in lieu of methanol.

To prepare the mixture:

1. Add 10 mL of 50:50 water:methanol to the methyl paraben bottle.
2. Cap the bottle and shake to dissolve.
3. Pour the contents of the methyl paraben bottle into the propyl paraben bottle.
4. Cap the bottle and shake to dissolve.

Now, the mixture is ready for use.

> ☑ ***Note***
> These compounds may not show up with an ELSD detector due to their volatility.

Verification Method

The verification method assumes the use of a 5 mL sample loop with a Redi*Sep*® Prep C18 20 x 150 mm 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. Open the ParabenVerification.pmtd method using the FILE | OPEN commands.
   - If this method file is not loaded on the ACCQ*Prep*, see the method parameters at the end of these instructions.
2. Choose your appropriate solvents. Either methanol or acetonitrile can be used as the B solvent.
3. Press PLAY.
4. Select the injection method appropriate for the system, programming the system for a 1 mL injection, then press then press START EQUILIBRATION.
5. If performing a manual injection:

- Choose LOAD AFTER EQUILIBRATION
  After equilibration and when prompted, inject 1 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.

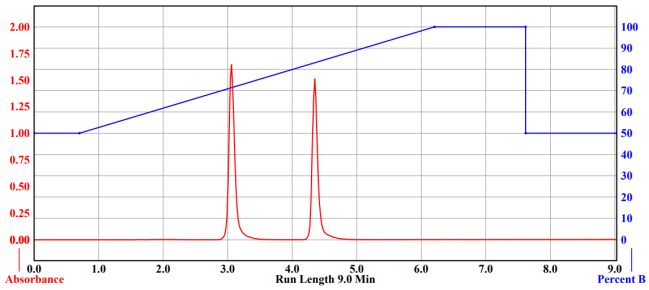

Figure 1: Water:Acetonitrile result

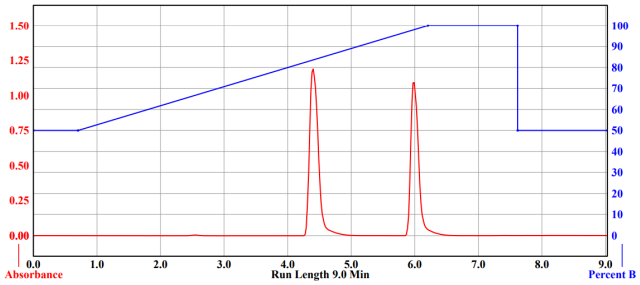

Figure 2: Water:Methanol result

6. Typical results are shown in Figures 1 and 2

Acceptance Criteria

Acetonitrile	
Peak 1	3.1 ± 0.2 min
Peak 2	4.4 ± 0.2 min
Methanol	
Peak 1	4.4 ± 0.2 min
Peak 2	6.0 ± 0.2 min

Troubleshooting

1. Peaks do not match the graph.
   a. 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.
   b. Retention time may vary due to the presence of modifiers such as TFA, differences between columns, unknown solvents remaining in col-

umn before separation started, insufficient injection loop flushing (manual injection only), etc.

2. Peaks elute too early.
   a. Using a column other than a Redi*Sep* Prep C18 20 x 150 mm.
   b. 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.
   c. 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.
   d. Loop not flushed before injection (manual injection only).
3. Peaks elute too late.
   a. Using a column other than a Redi*Sep* Prep C18 20 x 150mm.
   b. Check flow rate (collect fractions and inspect for correct volume). May need to reprime the pumps due to air in pump heads.
   c. Using a 20 x 250 mm column will cause peaks to elute ~67% later and will have a wider retention time variation.
   d. An ACCQ*Prep* installed with an ELSD or MS may have slightly delayed retention times due to additional plumbing.
   e. 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.
4. Peak 1 wider than peak 2.
   a. Injection loop not properly flushed (manual injection only).
5. No peaks or peaks very small.
   a. Sample injected before the play button is pressed and within 1 minute of previous separation (manual injection only).
   b. Rotor in injection valve assembled incorrectly.

Prep Verification Method

Redi*Sep* Prep C18 20 x 150 mm Equilibration volume: 90 mL

Flow rate: 18.9 mL/min

Fraction Collection: Peaks only

Gradient:

Duration (Minutes)	%B
0	50
0.7	50
5.5	100
1.4	100
0	50
1.4	50

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