



Efficient Conversion of HPLC Instruments between Normal-Phase and Reversed-Phase Solvents

Richard Henry and Carmen T. Santasania
carmen.santasania@sial.com

While reversed-phase (RP) HPLC is still by far the most common mode, normal-phase (NP) HPLC is increasing in popularity with the introduction of new highly polar columns with excellent retention, selectivity and stability. It is easy to inter-convert between RP and HILIC (a type of NP) because both modes employ polar aqueous mobile phases; however, many compounds are not polar enough to be retained under aqueous HILIC conditions. When aqueous solvents must be replaced with non-aqueous conditions to study non-polar samples, immiscibility situations can arise during changeover.

Customers often contact our Technical Service Department with a practical question that might go something like, "I need to change my HPLC instrument from RP mode to NP mode. Do you have any suggestions or guidelines for such a changeover?" This article describes our best practices for converting between reversed-phase and normal phase solvents, which are often immiscible.

The first best practice is to dedicate instruments to a specific mode. Significant seal wear-and-tear can be caused by expansion, contraction and extra friction of changing solvents. If possible, columns should also be dedicated to one mode for trouble-free operation. If dedicating the instrument or column is not possible, one should use the following procedure.

Our regular practice is to replace the column with tubing or a union and flush extensively with isopropanol (IPA) before going over to water or hydrocarbon. Before beginning the

changeover process, remove the HPLC column, cap and store in the appropriate storage solvent unless the same column is to be used in the new mode. Columns such as Cyano and Fluorophenyl (F5) can work in either RP or NP mode and can remain installed if desired. After IPA flush, the column can be removed and capped to avoid excessive wear on the valuable component. In the flushing steps, be sure to include the entire fluid path (pump, autosampler, valves, detector, etc.). Also, include the sample loop and any other fluid paths that are encountered for the normal operation of making injections. This can vary considerably depending on whether the autosampler is an external loop design, or an internal loop design. As part of all the washes, make certain the injection needle gets washed as well. It is best to do several full loop injections of a solvent such as IPA that is miscible with both high aqueous and high organic mobile phases. The total volume of IPA needed will vary with instrument design, but the waste volume should be monitored (record the volume as a guide for future changeovers) and observed for uniform appearance. UV detectors may remain on (ca. 250 nm) during this step to indicate when the system has returned to a stable baseline.

The second wash step after the IPA should be with methanol (or ethanol). Follow the previous procedure that was used for the IPA wash before going to water. Methanol will help flush the IPA out faster than going directly from IPA to water. If excessive baseline noise or drift is observed with a UV detector, repeat the procedures and allow more time to flush out any poorly swept flow regions.

Table 1. Properties of Organic Solvents Commonly Used in HPLC

Solvent	Polarity	Miscible with Water?	UV Cutoff*	Refractive Index at 20 °C	Solvent Strength e° (silica)	Viscosity at 20 °C, C P	
Hexane	nonpolar	no	200	1.3750	0.00	0.33	
Isooctane	↓	no	200	1.3910	0.01	0.50	
Carbon tetrachloride		no	263	1.4595	0.14	0.97	
Chloroform		no	245	1.4460	0.31	0.57	
Methylene chloride		no	235	1.4240	0.32	0.44	
Tetrahydrofuran		yes	215	1.4070	0.35	0.55	
Diethyl ether		no	215	1.3530	0.29	0.23	
Acetone		yes	330	1.3590	0.43	0.32	
Ethyl acetate		poorly	260	1.3720	0.45	0.45	
Dioxane		yes	215	1.4220	0.49	1.54	
Acetonitrile		yes	190	1.3440	0.50	0.37	
2-Propanol		yes	210	1.3770	0.63	2.30	
Methanol		yes	205	1.3290	0.73	0.60	
Water		polar	yes	-	1.3328	>0.73	1.00

* typical values.



Some Dos and Don'ts for Solvent Changeover:

- Do remove all additives and start with 100% isopropanol in all reservoirs.
- Isopropanol is fully miscible with all common solvents and is the safest changeover solvent for either direction.
- Do use low flow - about half of normal to avoid excessive seal wear and damage due to over-pressuring.
- Don't use acetonitrile routinely as the changeover solvent- it is better than methanol, but is not fully miscible with pure hydrocarbons.
- Don't use methanol routinely as the organic- it is not fully miscible with many normal phase conditions.
- Either acetonitrile or methanol may be used to routinely change from reversed-phase and back (remember to remove additives).
- Do check miscibility (use small external vessel) with target mobile phase before starting, especially if IPA is not selected.
- Do use organic (such as IPA) in all lines of a gradient instrument to make certain that water or hydrocarbon is removed from all fluid areas.
- Do operate the injector valve and any other selector valves while doing the IPA flush procedure.
- Do monitor pressure and detector signals during changeover as these are excellent methods to confirm full system equilibration; evaporative detectors such as MS and ELSD cannot be used for this purpose.
- Incomplete mixing shows up as severe detector baseline noise or pressure fluctuations (globules of immiscible solvent can resemble bubbles or particles).
- Do flush detectors and all other components even if baseline is not monitored.
- Total time for changeover can vary but should take about an hour. Do not rush; this may actually slow down the process.
- Do record the volumes of solvent used during changeover for use as a future guide; if changeover is unsuccessful, use more solvent the next time.
- Don't expect fast changeover and baseline equilibration with refractive index detectors- they are extremely slow to equilibrate after changeover.
- Do check gradient blank runs for excessive baseline noise and drift that might indicate pockets of immiscible solvent.
- Good chromatography in the target mode is the most sensitive final test- start with simple binary mobile phases and standard test mixes and work toward real samples with mobile phase additives.

Finally, it is also good practice to contact your LC instrument manufacturer to be sure all details of the changeover are covered. The manufacturer may have additional details and tips for successful changeover to a different chromatographic mode. If columns and instruments are frequently used in different modes, adopt a labeling system to alert a new user about possible solvent compatibility issues.



All the Great HPLC Brands Under One Roof

- Supelco®
- TSK (Tosoh)
- Kromasil®
- Hamilton®
- Upchurch®
- Rheodyne®
- Spherisorb®
- and more...*

Find the products you need, including pricing and availability at sigma-aldrich.com/hplc

Purchase any of these products at a **20% Discount** through Dec 31, 2010. (Supelco and TSK not included) Specify Promo Code 425 when ordering.