MICROSOLV
TECHNOLOGY CORPORATION

# White Paper

## Using Aqueous Normal Phase to Your Advantage

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## Using Aqueous Normal Phase to Your Advantage

Using HPLC for polar compounds can be problematic with traditional separations modes such as reverse phase. Now, you can retain and separate polar compounds using aqueous solvents at high organic strength for these compounds without extremes of pH or temperature.

Using Aqueous Normal Phase you can elute and retain compounds by normal phase mode using reverse phase solvents. This will allow you to retain and elute polar and non polar compounds in a single, isocratic run.

Expand the useful range of sample polarity in HPLC.



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## Traditional HPLC

Normal Phase is normally used for polar solutes.

Traditionally HPLC is divided into two categories: normal phase and reversed phase. In normal phase chromatography the stationary phase is polar and the mobile phase is non-polar. In reversed phase we have just the opposite; the stationary phase is non-polar and the mobile phase is polar. Typical stationary phases for normal phase chromatography are silica or organic moieties with cyano and amino functional groups. For reversed phase alkyl hydrocarbons are preferred with the most common being octadecyl (C18) but with octyl (C8) and butyl (C4) also used in some applications. The designations for the reversed phase materials refer to the length of the hydrocarbon chain. Therefore, the elution order in normal phase chromatography is as follows: the most non-polar compounds elute first and the most polar compounds elute last. Conversely, in reversed phase, the most polar compounds elute first and the most non-polar compounds elute last. Normal phase chromatography is used for the analysis of polar solutes such as amines, acids, metal complexes, etc. Whereas, reversed phase is typically used for less polar species and those with some hydrophobic properties.

## Two Modes With Different Solvent Systems

Reverse Phase is normally used for non polar solutes.

In reversed phase chromatography the mobile phase is generally a binary mixture of water and a miscible polar organic solvent like methanol, acetonitrile or THF. Retention increases as the amount of the polar solvent (water) in the mobile phase increases. In normal phase the mobile phase consists of a very non-polar solvent like hexane or heptane mixed with a slightly more polar solvent like isopropanol, ethyl acetate or chloroform. Retention increases as the amount of non-polar solvent in the mobile phase increases. Switching back and forth between these two systems is time consuming and not efficient. Therefore most laboratories attempt to retain and separate polar compounds in reverse phase using atypical methodologies such as pH extreme or additives.

## A Third Mode Solves This Dilemma

A Third Type of Chromatography has been developed.

Recently a third type of chromatography has been developed with stationary phases based on silica-hydride surfaces. It is referred to as aqueous normal phase chromatography (ANP). The principle of ANP is simple. Retention behavior is analogous to that found in normal phase chromatography but the mobile phase has some water as part of the binary solvent. Normal phase implies that retention is greatest for polar solutes such as acids and bases. In addition, retention must increase as the amount of the non- polar solvent in the mobile phase increases. So if the mobile phase consists of water and acetonitrile, retention will increase as the amount of acetonitrile increases. Typically the amount of the non-polar component in the mobile phase must be 60% or greater with the exact point of increased retention depending on the solute and the organic component of the mobile phase.



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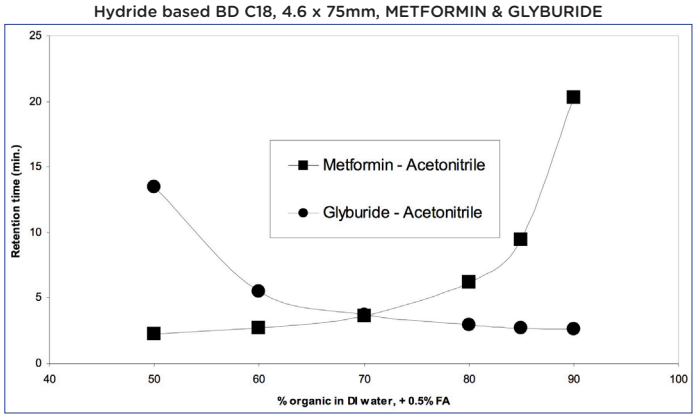
# A Real Life Example of Aqueous Normal Phase

Aqueous Normal Phase is used for polar and non-polar solutes at the same time.

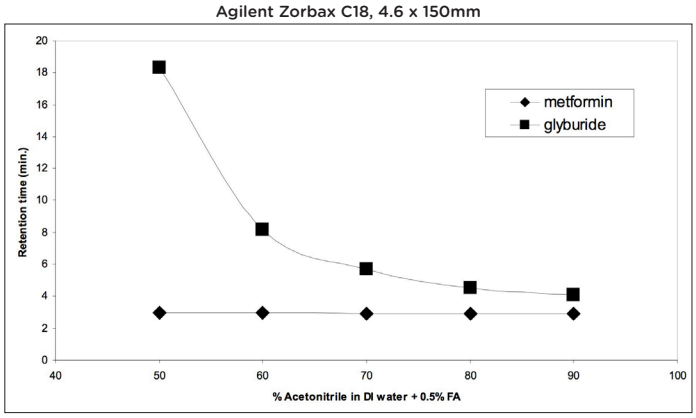
A comparison of a compound with only reversed phase behavior (the non-polar molecule Glyburide) to one with ANP retention (the highly polar compound metformin) is shown in the first figure on the left below. For Glyburide (●), retention decreases as the amount of acetonitrile increases in the mobile phase; typical reversed phase behavior. For metformin (■), retention increases as the amount of acetonitrile is added to the mobile phase; typical normal phase behavior but with an aqueous component in the mobile phase, hence the designation aqueous normal phase. An interesting feature of this plot is that for acetonitrile it is possible for the highly polar compound metformin and the non-polar compound Glyburide to co-elute with both having reasonable retention.

This co-elution would occur at 70% acetonitrile with a retention time of over four minutes. Their results can be compared to the graph on the right for a typical commercial C18 column. Only reversed phase behavior is observed for the non-polar compound Glyburide. Similar ANP retention has also been demonstrated for other polar compounds on the hydride based stationary phases. The dual retention mechanisms of reversed phase and aqueous normal phase is shown in the separation below. A group of both polar and non-polar compounds are retained and separated under isocratic conditions.

Polar and Non-polar Compounds on a Cogent TYPE-C HPLC Column.




Polar and Non-polar Compounds on a Leading Traditional HPLC Column.



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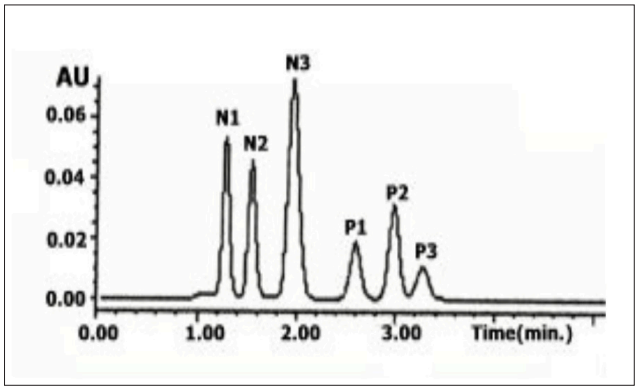
## Use Mass Spec Compatible Mobile Phases as Well

Another important feature of the TYPE-C phases is that for many analyses it is usually not necessary to use a high pH mobile phase to analyze polar compounds such as bases. In the example below, the aqueous component of the mobile phase contained 0.5% formic acid (FA) which is very compatible for mass spectral analysis.

Nucleoside Analogs: Anticancer Prodrugs by HPLC




**Mobile phase: 60:40 acetonitrile/DI water + 0.5% formic acid**

**Samples: Proprietary drugs: N1 – N3 nucleoside analogs, P1 – P3 other more hydrophobic proprietary compounds with polar functional groups**

## Where To Get These Columns

Available Worldwide

Aqueous Normal Phase is available on all Cogent TYPE-C™ silica based HPLC Columns from MicroSolv Technology Corporation and our international dealers. Visit our website or call for more information on how to purchase these columns.



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