Special Mobile Phase Considerations for ZirChrom®-CARB and Diamondbond®-C18

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Special consideration must be taken when choosing an organic modifier additive for use with carbon-clad reversed phase materials (i.e. ZirChrom®-CARB and DiamondBond®-C18), particularly for separations involving strongly retained analytes such as highly conjugated aromatic or planar molecules. This research presents several alternatives to traditional strong HPLC solvents. The results suggest that the strongest solvent additive available should be used so that the least amount of additive will be required to achieve the desired retention, thereby allowing the smallest background absorbance due to the mobile phase.

Introduction

Carbon-based HPLC stationary phases are very different from bonded phase reversed-phase materials, especially in terms of chromatographic selectivity and mobile phase considerations. This article explores the key considerations important to unlocking the power of these very different columns from the mobile phase perspective. In particular we show here that mobile phase constituents have a marked effect on retention of analytes on carbon-based columns, (i.e. ZirChrom®-CARB and DiamondBond®-C18), particularly for separations involving strongly retained analytes such as highly conjugated aromatic or planar molecules. Method development issues such as peak tailing, low column efficiency, and irreversible adsorption can often be overcome with the inclusion of a relatively small amount (1 -10%) of a very strong solvent additive in the organic portion of the mobile phase. Widely employed "strong" HPLC solvents such as tetrahydrofuran (THF) are extremely effective mobile phase additives for carbon phases, however, they also have some limitations including: high absorbance at low UV wavelengths, incompatibility with PEEK tubing, peroxide formation, and general toxicity. In an effort to discover alternatives, Professor Carr et al., at the University of Minnesota, systematically have studied the use of small amounts (0.1-10% (v/v)) of aliphatic alcohols, nitriles, and amines of increasing chain length as mobile phase additives to improve the chromatographic performance of strongly retained compounds (in this case steroidal compounds) on ZirChrom's carbon phases. Their findings are summarized here.

Experimental

A baseline mobile phase of 70/30 ACN/water was used with a 100 mm x 4.6 mm i.d. ZirChrom®-CARB column at 80 ^{o}C, and the retention factor of three anabolic steroids were measured as a function of the amount and type of mobile phase constituent used. For example, to test the effect of 1% 1-octanol, a fraction of the ACN was replaced with the 1% 1-octanol such that the total volume fraction of organic modifier remained the same (i.e. 69/1/30 ACN/1-octanol/water).

Column:	ZirChrom®-CARB, 100 mm x 4.6 mm i.d. (Part Number: ZR01-1046)
Mobile Phase:	As indicated.
Temperature:	80 ºC with Metalox™ 200-C column heater

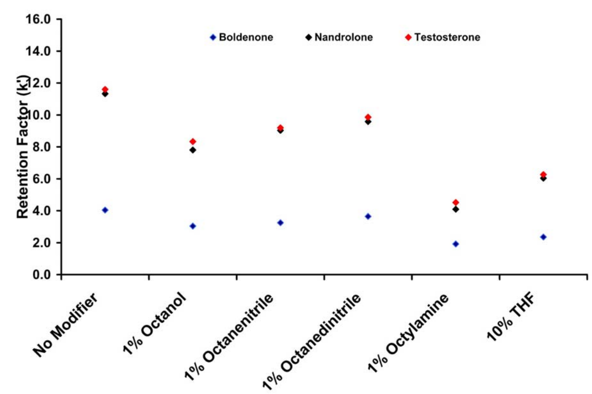

Figure 1. Effect of 8-carbon mobile phase additives.

Figure 1 shows the effect of adding only 1% of several different 8-carbon additives (octanol, octanenitrile, octanedinitrile, and octylamine) on the retention of the three steroids, in comparison to the 70/30 ACN/water mobile phase alone, and the use of 10% THF (i.e. 60/10/30 ACN/THF/water). These data show that even small amounts of these 8-carbon additives have a profound effect on the retention of highly retained compounds.

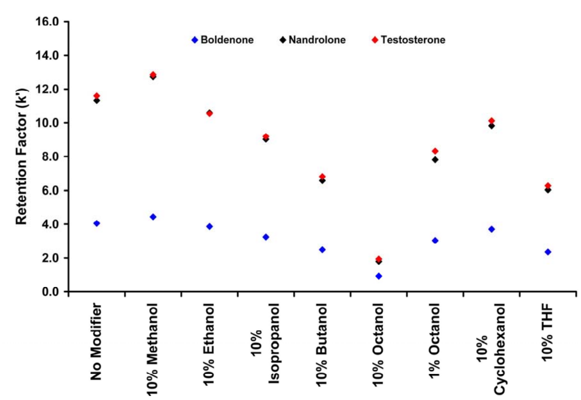

Figure 2. Effect of alcohol mobile phase additives.

Figures 2-4 show that as the length of the alcohol, nitrile, or amine modifier that is used is increased, the retention of the steroidal probe solutes decreases. In addition, Figures 2-4 suggest that a broad range of solvent strength is available through the use of relatively simple additives such as octanol or butanol. In general, it was also observed that column efficiency did improve with increasing chain length of the modifier. When working with UV detection, it is important to be mindful of the background absorbance of the mobile phase, particularly when working with low UV wavelengths (see Figures 5-6).

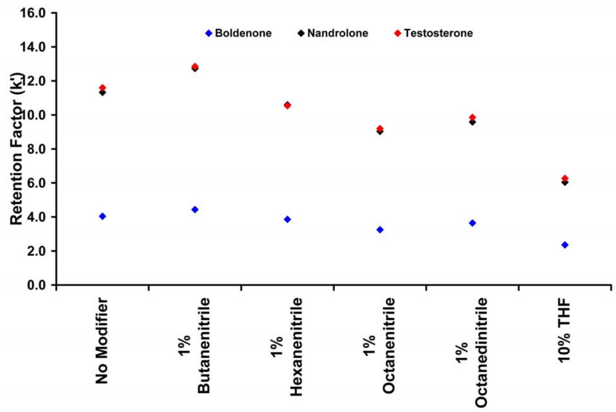

Figure 3. Effect of nitrile mobile phase additives.

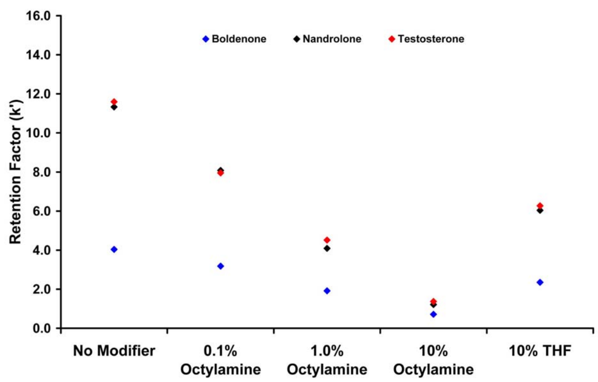

Figure 4. Effect of concentration for octylamine mobile phase additive.

Figures 5 and 6 show UV spectra in the wavelength range of 200-300 nm for mixtures of all of the additives compared in this work, where each mixture consisted of 65% ACN, 30% water, and 5% (v/v) of the indicated additive. Figure 5 focuses on mixtures that were available in HPLC-solvent grade purity. Figure 6 compares three HPLC-solvent grade purity mixtures (ACN, THF and MTBE) to three other mixtures not available in HPLC-solvent grade purity.

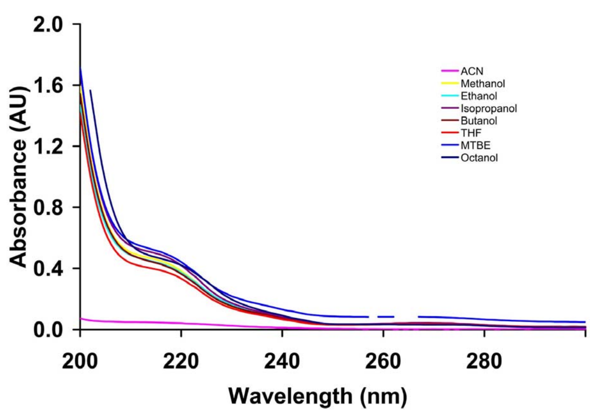

Figure 5. UV spectra of mobile phase additives.

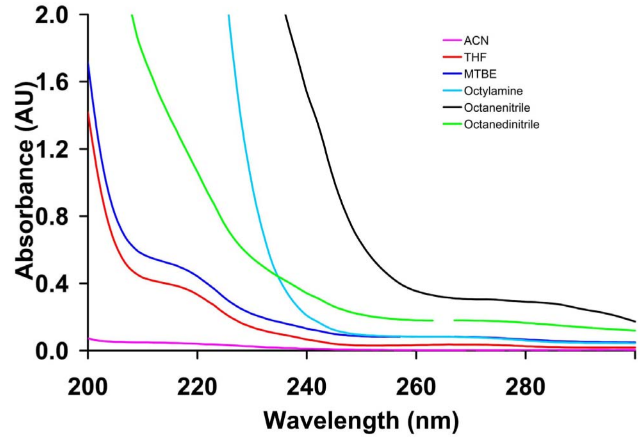

Figure 6. UV spectra of mobile phase additives.

Summary

In summary the use of simple mobile phase additives such as THF, butanol, octanol or octylamine are very useful for reducing retention of strongly interacting solutes on carbon phases and in general can be used to improve chromatographic efficiency and peak shapes on carbon-based HPLC columns.

This research can be tailored to your specific application needs. ZirChrom technical support can help to optimize and transfer a your method with a ZirChrom carbon column. Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for details.

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