What determines the retention of a molecule in reversed phase HPLC – FAQ

There has been much research and work done in this area and many books have been written about this topic.

However, to give a very short answer you could say that much of the retention of a column in **Reversed Phase RP** HPLC is due to what is commonly called hydrophobicity. This natural phenomena is due mostly to the size of the hydrophobic (water resistant) area of a molecule. Retention will increase with the amount of water in the mobile phase. In general, the more hydrophobic the molecule the longer it should be retained.

If a molecule is “solvo-phobic” the molecule will retain elsewhere, namely the column. This goes to the old adage in chromatography “like likes like”.

NOTE: This does not apply to Normal Phase HPLC, Aqueous Normal Phase ANP or Ion Exchange Chromatography.

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MicroSolv Technology Corporation
9158 Industrial Blvd. NE, Leland, NC 28451
tel. (732) 380-8900, fax (910) 769-9435
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Website: www.mtc-usa.com
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