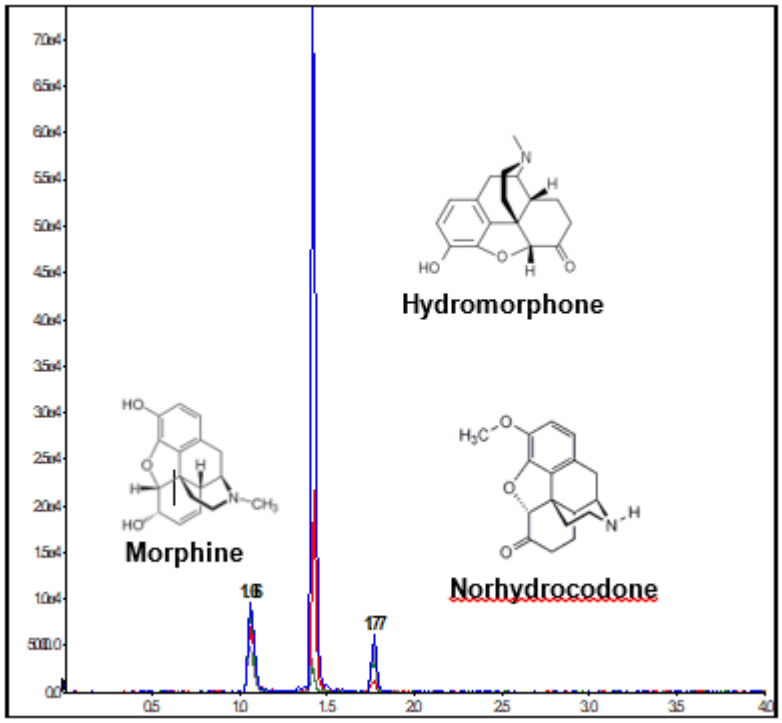
The danger of mobile phase mismatch

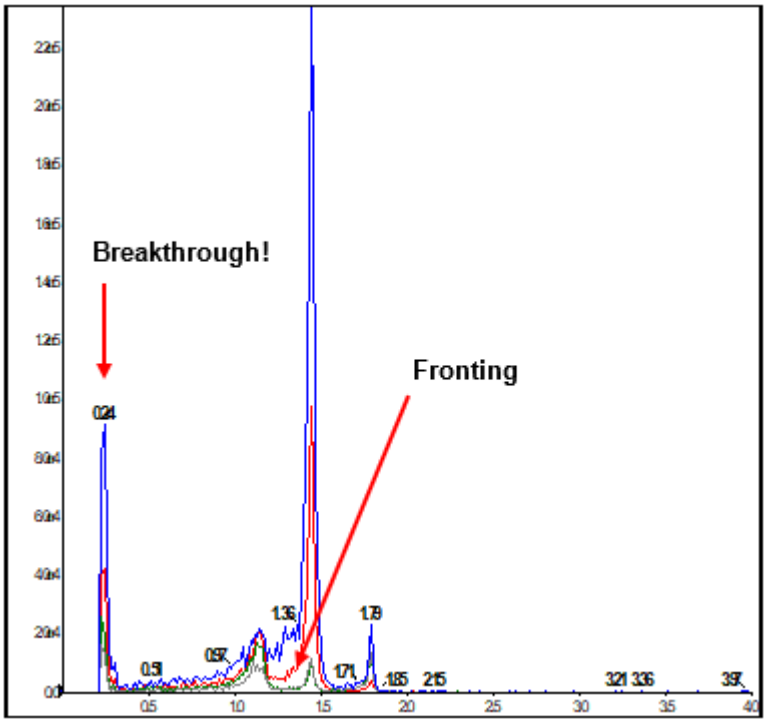
It is easy to think that once a sample elutes from Solid Phase Extraction (SPE) it is then ready to be injected onto the analytical column. While the sample is now significantly cleaner, it is not always the case that injecting it directly onto the column will result in perfect chromatography. Solvent mismatch is a known cause of poor peak shape, so care must be given to consider the solvent strength used in the elution step of any SPE being performed on samples.

The solvent with which the sample is eluted, the overall concentration of the sample, and the mobile phase conditions of the method should be evaluated when building sample preparation protocols. An injection of 100% organic solvent when your starting Mobile Phase conditions begin with high water (90%), can cause solvent mis-match to occur within the system, as seen in Image 1 below. Ideally, a sample will be in solvent that closely matches the starting mobile phase ratio of the LC method to prevent issues in peak shape from occurring.

Remember that not all elution solvent ratios are troublesome to chromatography. A good example of a favourable elution solvent is when Phree™ Protein Precipitation product is used to prepare samples for analysis in HILIC (hydrophilic interaction liquid chromatography) mode. The Phree protocol calls for samples to be diluted in a 3 : 1 (Acetonitrile : Aqueous) based sample ratio for the protein precipitation step to be successful. HILIC mode starts at high concentrations of Acetonitrile, but still requires water to be present in the sample for optimal peak shape. The sample eluting from Phree is already in the ideal solvent ratio for HILIC mode and no further processing is needed, but if your analysis is looking to use Ion Exchange with Rezex™, the presence of Acetonitrile in the sample could cause issues.



Sample in 50% Methanol



Sample in 100% Methanol