

Obtain a flat baseline in a gradient method without using blank subtraction – How to

There is another way you can obtain a flat baseline besides “blank subtraction” but it can be a little tricky. It is called “*absorbance matching*”.

Typically when your B solvent is mostly acetonitrile, it will absorb more than the A solvent because of the acetonitrile. What you need to do is add an appropriate amount of a UV-absorbing **additive** to the A solvent such that the two solvents absorb the same at your wavelength.

The **additive** should be un-retained and should not interact with or affect the sample. Examples include nitrate, nitrite, azide compounds, etc. Determining the proper amount to add can be done by trial and error. When the two solvents are matched evenly, there will be no change in the baseline regardless of the gradient.

Reference: L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, 2nd Ed, 1997, John Wiley & Sons, pg. 396.