Pre mix your diluent and mobile phase in sample preparation for HPLC – Tips & Suggestions

Suppose your method calls for a diluent of 50% DI water/ 50% acetonitrile. You are making a 1 mg/mL stock solution and have added 100.0 mg reference standard to a 100 mL volumetric flask. Would it be acceptable to add 50.0 mL DI water and then dilute to mark with acetonitrile?

While this approach may seem fine at first consideration, it does not account for the volume change due to mixing these two solvents. Hence, when you dilute to mark and invert your solution to facilitate dissolution of the reference standard, you are also mixing the two solvent components that comprise the diluent. Hence, your concentration will be off due to the inaccurate mixed volume.

A better way is to prepare your 50% DI water/ 50% acetonitrile diluent ahead of time, separately. Once it has been mixed, you can add it to your flask with the reference standard, and there will be no volume change due to mixing in this case.