

UV-Vis Lesson 4 - Preparing your Sample and Evaluating Your Result

The UV-Vis spectrum will be obtained from a HPLC-PDA instrument. PDA stands for PhotoDiodeArray and is the detector used on the HPLC system. A PDA detector allows you to obtain the entire UV-Vis spectrum of a compound as it comes off of the HPLC column.

The HPLC-PDA is similar to the GC-MS. The sample is separated by HPLC chromatography into its component parts and then a UV-Vis spectrum of each compound in the original sample is obtained.

The HPLC sample should have the same concentration as the GC-MS sample (*i.e.* 0.02 grams analyte / 10 mL solvent). However, for HPLC, the solvent should be methanol or acetonitrile. Other solvents, like CH₂Cl₂, can interfere with the HPLC separation. If the BP of the analyte is less than 140°C, then you can use methanol or acetonitrile as GC-MS solvents also. In those cases, the solution you prepare for GC-MS can also be used for HPLC-PDA sample analysis.

The solution for analysis can be prepared in two different ways.

- (1) For *solid* (or liquid) *compounds*, dissolve 0.02 grams analyte into 10 mL of methanol or acetonitrile. Swirl or mix the solution with a new, clean pipette to dissolve the analyte and obtain a homogeneous solution. Then, because this is an HPLC sample, filter the analyte solution through a small cotton plug in a disposable pipette before using it for sample submission.

Or

- (2) For *liquid compounds* dissolve 4 µL (using the GC syringe) of the analyte into 2 mL of methanol or acetonitrile. Mix the solution with a new, clean pipette to dissolve the analyte and obtain a homogeneous solution. If you need 4 mL of analyte solution, you can mix 8 µL of the analyte with 4 mL of methanol or acetonitrile.

<p>QUVVis4-1. How concentrated in terms of g / mL of a solid analyte or μL / mL for a liquid analyte should the sample be for HPLC-PDA analysis?</p>	
<p>QUVVis4-2. List reasons why is methanol (or acetonitrile) a good solvent for HPLC-PDA analysis.</p>	<p>AUVVis4-1. 0.02 g analyte / 10 mL solvent for solids 4 μL analyte / 2 mL solvent for liquids</p>
<p>QUVVis4-3. If you are going to prepare a sample of a compound (BP > 140°C) for both UV-Vis and GC-MS analysis, can you use the same sample solution for both GC-MS and UV-Vis analysis?</p>	<p>AUVVis4-2. Methanol dissolves most organic compounds. Methanol does not contain an active π bond chromophore. The lone pair in methanol is excited at 183 nm. Methanol will not interfere with the reverse phase HPLC separation.</p> <p>Ditto for acetonitrile. Despite the <i>nonconjugated</i> pi bonds in acetonitrile, it is transparent in UV-Vis spectral range.</p>
	<p>AUVVis4-3. Yes. Solvent choice should be methanol or acetonitrile.</p>