

SAMPLE PREPARATION GUIDE - PROTEINS & PEPTIDES

SOLVENT REQUIREMENT FOR ELECTROSPRAY MASS SPECTROMETRY

This information is intended to provide guidelines for the formulation of samples to be analyzed by mass spectrometry. Basically it is a general list of sample components to include and to avoid in preparing samples for a typical MS analysis. If you cannot meet these standard formulation conditions for any reason, please discuss any special sample requirements with the Facility Manager. Together we can map out a strategy for optimal sample handling and preparation.

Mass spectrometry cares only about the mass of your protein / peptide; it is independent of the correct fold! You can sacrifice the activity for this type of biophysical analysis.

Solvent components for direct injection = 50% H₂O + 50% Organic Solvent + 0.1% Organic Acid

H₂O – distilled and deionized water

Organic Solvent – typically acetonitrile since it is commonly used in reverse phase HPLC, but many other organic solvents will suffice: methanol, ethanol, n-propanol, isopropanol, etc. HPLC grade solvents are best!

The ratio between H₂O and organic is not critical, 1:1 is typical. An organic solvent is added for two reasons:

1. to increase the solubility of the analyte(s)
2. to promote desolvation of the charged droplets formed by the electrospray process

Organic Acid – a dilute organic acid is used to ensure protonation of the analyte(s) so that their positive ions can be observed. Strong mineral acids such as HCl and H₂SO₄ are typically not used as their counterions can form strong ion pairs with the positively charged analyte(s), decreasing sensitivity. The most commonly employed acids are:

trifluoroacetic acid (TFA) – pK_a 0.30

formic acid – pK_a 3.75

acetic acid – pK_a 4.75

Since TFA is widely used in reverse phase HPLC, it is also frequently used in mass spec. However due to its very low pK_a, the abundant trifluoroacetate ion can form strong ion pairs with positively charged analyte(s), decreasing sensitivity. For this reason, TFA should be avoided in samples with a very low concentration of analyte(s), e.g. subpicomolar.

Thus the typical direct injection mass spec sample is dissolved in 50:50:0.1 H₂O:ACN:TFA unless it is a very low level sample in which case acetic acid or formic acid are substituted for the TFA.

Since most final formulations of proteins typically contain salt and/or buffers, a good alternative for direct injection (no HPLC) of proteins is final formulation in or buffer exchange into dilute NH₄HCO₃ (10-100 mM). Ammonium bicarbonate sublimates into ammonia, water, and carbon dioxide during lyophilization. You can then submit your samples as a nearly salt-free lyophilized solid.

Your sample may contain the following, but we will have to do HPLC

Mass spectrometry is very sensitive. Remember that many other compounds besides what you are interested in (protein, peptide) can be analyzed. For this reason, your samples must be very "clean". If you have any of the following, they must be removed by HPLC prior to entering the mass spectrometer.

SALTS: the sample must not contain any salts such as NaCl, K₂HPO₄, etc. The positive ions (Na⁺, K⁺, Ca²⁺) can substitute for H⁺ in the analyte, especially at acidic sites, and greatly complicate interpretation of the spectra. Negative ions (Cl⁻, SO₄²⁻) can form ion pairs with basic sites and neutralize positive charge in the analyte(s). Furthermore, positive salt ions can be reduced in the mass spectrometer and plate out on the ion optics, damaging the instrument.

BUFFERS: the sample must not contain any organic buffer salts such as TRIS, MES, MOPS, etc. for the same reasons listed above. This also includes reducing agents such as BME and DTT.

COMPONENTS YOU MUST AVOID!!

You MUST completely avoid all of the following components. They are very difficult and in some cases impossible to remove with HPLC.

STABILIZERS: the sample must not contain any glycerol, PEG or related stabilizers. These components ionize particularly well, especially PEG, and if they are present, that is all you will see! Also, these components can be difficult to get rid of once your protein or peptide has seen them so they should even be avoided during sample preparation or used only in very early steps of purification.

DETERGENTS: the sample must not contain either ionic (e.g. SDS) or non-ionic (e.g. Triton X-100) detergents. Detergents ionize particularly well and if they are present, that is all you will see! Also, these components are typically very hard to get rid of once your protein or peptide has seen them so they should even be avoided during sample preparation or used only in very early steps of purification.

No colored eppendorf tubes!

These tubes often contain residual quantities of dyes. Since organic solvents are used in mass spec, the dyes can leach off the tubes into your samples. Especially if your sample is at low levels, the dyes can swamp out your signal! Use only clear plastic tubes. We can provide you with names of suppliers of "mass spec friendly" tubes.