

ESI sample preparation guidelines

Overview

0.5 – 2 mL in volume

1 – 5 μM in concentration

~ 50% 18M $\Omega\cdot\text{cm}$ deionized water + 50% HPLC grade Organic Solvent

Add ~0.1% Organic Acid to promote protonation in +ESI mode

FILTER TO REMOVE PARTICULATE MATTER

Concentration

Mass spectrometry is several orders of magnitude more sensitive to NMR, so please do not use the same samples you have prepared for NMR analysis for MS analysis. The upper limit of concentration needed is 5 μM . If you introduce a more concentrated sample, you will cause longterm contamination problems for other users. Additionally, in the short term, you will acquire less accurate data because the detector will be saturated and m/z shifts will occur.

WHEN IN DOUBT, DILUTE!

Aqueous/Organic Content

The ratio between water and organic solvent is not critical as long as water is < 80% of the solvent system. Purity of all solvents **IS** critical. Use high resistivity (18M $\Omega\cdot\text{cm}$) deionized water. Use good quality, **FILTERED**, HPLC grade (or better) solvents. Recommended organic solvents are methanol and acetonitrile. Nonpolar, nonprotic solvents do NOT make good ESI solvents.

Organic Acid

A dilute organic acid is used to ensure to 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 formic acid and acetic acid.

Sample Components to Avoid Completely

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). Salt clusters can dominate mass spectra and mask analyte signal. 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. Nonvolatile buffers such as phosphate buffers also must not be present in the sample.

Stabilizers and Detergents: the sample must not contain any **glycerol, PEG, SDS,** or related stabilizers or detergents. These components ionize particularly well, and will dominate mass spectra and mask analyte signal.