Since the advent of electrospray ionization in the early 1990s, mass spectrometry has become the leading analytical technology for quantitative and qualitative characterization of both endogenous and exogenous molecules in biological matrices. Three examples of the application of mass spectrometry to characterization of small molecules in biological matrices will be presented. These include: 1) Use of nanoelectrospray ionization for structure elucidation of drug metabolites in biological fluids. Nanoelectrospray exploits the concentration-sensitive nature of electrospray ionization to allow extended spectral data collection while consuming only microliter sample volumes. 2) High-resolution accurate-mass (HRAM) orbitrap mass spectrometry for metabolomics. HRAM is particularly well suited for metabolomics as it provides both relative quantitation and the mass accuracy necessary to yield elemental composition and, subsequently, structure identification. 3) Paper spray mass spectrometry for direct quantitation of xenobiotics in biofluids without sample preparation or chromatographic separation. Paper spray is one of the recently developed suite of ambient ionization technologies that enable direct interrogation of samples without the necessity of traditional sample preparation and cleanup.