The analytical challenges to acquire accurate isotopic data of intracellular metabolic intermediates for stationary, nonstationary, and dynamic metabolic flux analysis (MFA) are numerous. This work presents MID Max, a novel LC–MS/MS workflow, acquisition, and isotopomer deconvolution method for MFA that takes advantage of additional scan types that maximizes the number of mass isotopomer distributions (MIDs) that can be acquired in a given experiment. The analytical method was found to measure the MIDs of 97 metabolites, corresponding to 74 unique metabolite-fragment pairs (32 precursor spectra and 42 product spectra) with accuracy and precision. The compounds measured included metabolic intermediates in central carbohydrate metabolism and cofactors of peripheral metabolism (e.g., ATP). Using only a subset of the acquired MIDs, the method was found to improve the precision of flux estimations and number of resolved exchange fluxes for wild-type E. coli compared to traditional methods and previously published data sets.