

**Challenges and Solutions to the Bioanalysis of Large Molecules by LC-MS/MS:  
Development, Validation and Production**  
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Here we report on methods that improve the selectivity and sensitivity for the analysis of peptides and large molecules from biological fluids by LC-MS/MS. In-silico peptide selection, immunocapture, micro-elution solid phase extraction and microflow LC-MS/MS (MFLC-MS/MS) with optimal ESI source design were used to improve the selectivity, sensitivity and productivity for the analysis of large molecules from biological fluids. Limits of quantitation (LOQ) were improved by more than 10X and productivity was improved by more than 50%. These techniques are now in-place to routinely analyze thousands of samples per month in our laboratory.