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### **Challenges in peptide method development Interactions between peptides and HPLC vials**

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HPLC (High-Performance Liquid Chromatography) is a widely used analytical technique for separating, identifying and quantifying components in a mixture. Peptides are a class of biomolecules consisting of amino acids linked together by peptide bonds. Developing an HPLC method for peptides can present several challenges due to the unique characteristics of peptides. Peptides can vary significantly in terms of their physicochemical properties, they may have limited solubility and can be susceptible to degradation. Developing a method that provides adequate selectivity for the target peptides while minimizing interferences from matrix components can be demanding. Peptides can adsorb onto the surfaces of HPLC vials, which can lead to loss of sample and reduced chromatographic performance. Adsorption can occur due to interactions between the peptide and the vial material, such as glass or plastic, and can lead to reduced recovery of peptides, loss of sample and altered chromatographic behavior. Also, they may exhibit non-specific binding to the surfaces of HPLC vials, which can result in peak tailing and reduced sensitivity. Choosing vials made of materials that have minimal interaction with peptides, such as borosilicate glass or inert plastics like polypropylene, can help minimize adsorption and non-specific binding. Polypropylene is a widely used plastic material for pharmaceutical vials due to its favorable properties. Polypropylene vials offer excellent chemical resistance, low reactivity and good barrier properties against moisture and gases. Generally, vials are available in both clear and amber options, with amber vials providing enhanced protection against light-induced degradation. When considering amber vials, it is important to consider the potential interactions between the vial coating (such as a UV-blocking coating) and the peptide. It is crucial to evaluate different vial materials and optimize method parameters to mitigate peptide-vial interactions and develop reliable HPLC methods for peptide analysis.