Histidine Removal from Formulation Buffers Using HisSep™ Columns

Introduction:

Biologics, especially monoclonal antibodies (mAbs) and antibody drug conjugates (ADCs), have had a profound impact on medicine in recent years. mAb development has become a major focus in many major pharmaceutical and biotechnology companies. Approximately 70 mAb products are predicted to reach the worldwide market by 2020. Histidine is commonly used in biologics formulation buffers. However, histidine containing buffer interferes with certain bioanalytical methods, such as Amino Acid Analysis and Imaged Capillary Isoelectric Focusing (icIEF). In Amino Acid Analysis using HPLC, high concentration histidine may increase the amount of histidine in the final results, or consume too much labeling reagents, which may generate inaccurate results. In icIEF analysis, histidine produces a "histidine gap" or a "histidine dip" at a pI of about 7.5 in the electropherograms, and thus interferes with real sample peaks having pI 7.0 to 7.6, depending the ratio of the ampholytes. Therefore, removing histidine prior to these analyses is necessary in order to generate accurate results.

Currently histidine removal can be done by using molecular weight cut-off membrane spin columns. This type of spin columns can adsorb proteins on the membrane leading to unpredicted and disproportional loss of proteins. In addition, the need for high g-force, multiple spins with these spin columns adds substantial stress on samples which may introduce misleading information in stability studies and formulation development.

We have developed a spin column product, HisSep, optimized for the removal of histidine from biologics formulation buffers. Compared with current membrane-based spin columns, HisSep has significant benefits:

- **No additional stress placed on the proteins in solution**
- **Rapid 4 minute process vs. up to 20 minute process**
- **Greater than 90% recovery**
- **Proteins remain in stable buffer medium**
- **Compatible with most downstream analysis**

Applications: Histidine removal from ADC and mAb samples in formulation buffer

An ADC and a therapeutic mAb were formulated in a formulation buffer containing 20 mM histidine. The charge variants of the ADC and the mAb were analyzed using icIEF on iCE280. Before removal of histidine, a "histidine gap" in the ADC sample and a "histidine dip" in the mAb sample are observed around pI 7.0 (Fig. 1A and 2A). The histidine gap and the histidine dip interfere with the icIEF profiles of the ADC and the mAb. The histidine gap and dip were completely removed after histidine removal using HisSep™ columns (Fig. 1B and 2B).

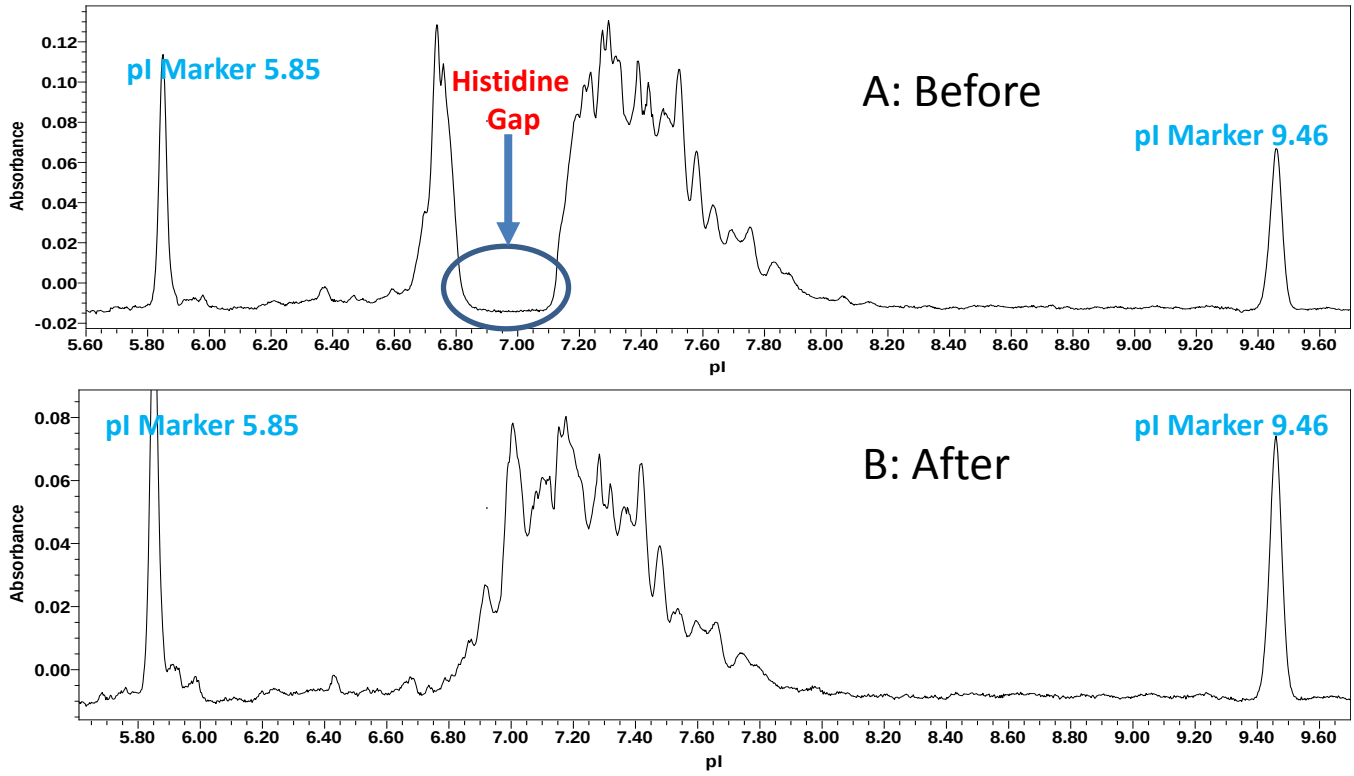

Fig. 1: Electropherograms of an ADC sample in formulation buffer, A: before histidine removal; B: after removal of histidine using a HisSep™ column. Sample recovery: 91%.

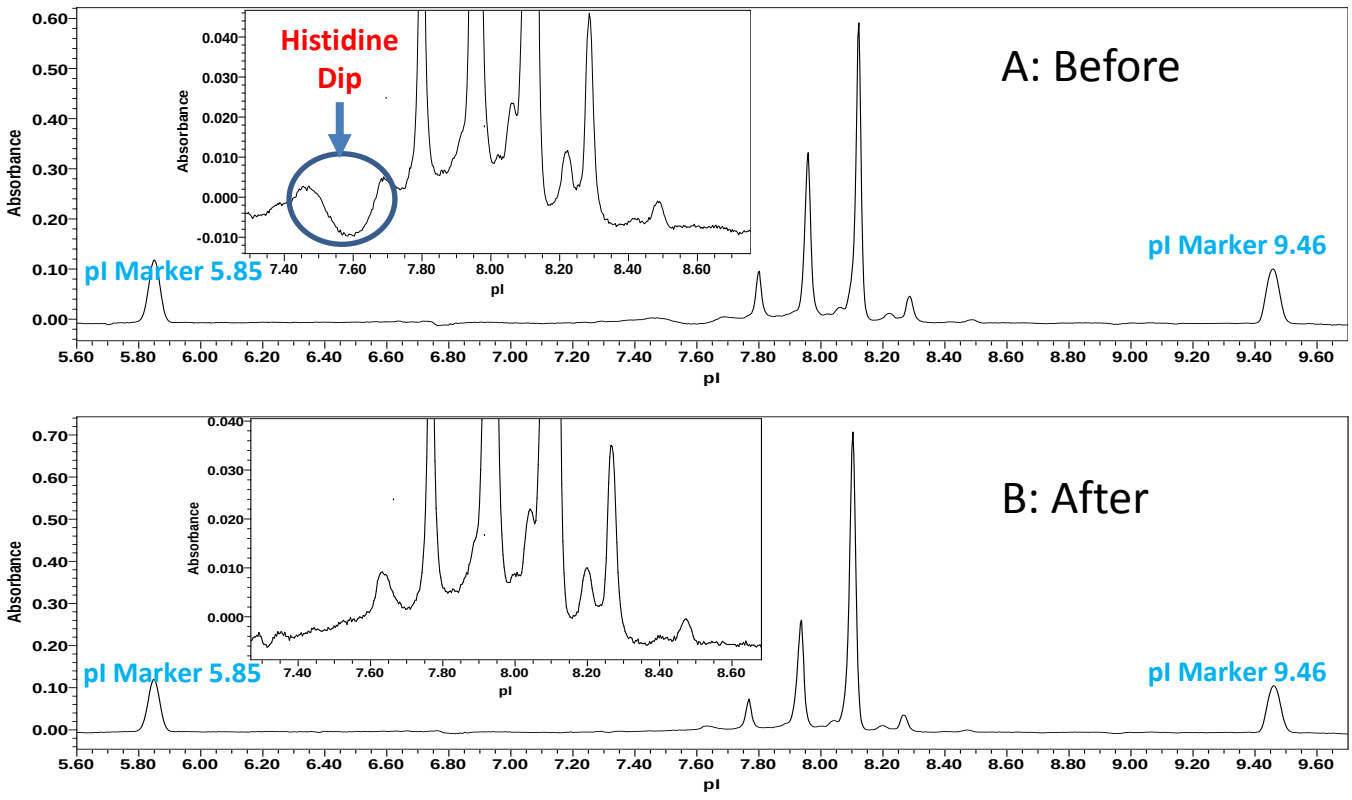

Fig. 2: Electropherograms of an mAb sample in formulation buffer, A: before histidine removal; B: after removal of histidine using a HisSep™ column. Sample recovery: 96%.