



Desalting and Concentrating Biologics in One Single Easy Step Using ConSep™ Column

Introduction:

Biologics, especially monoclonal antibodies (mAb) and antibody drug conjugates (ADC), 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. High salt and low drug substance concentrations in biologics during the development phase pose huge challenges for analytical work flow, which may limit accurate measurement of some important attributes of the biologics. Some of the commonly used analytical techniques in biologics development, such as CE-SDS and icIEF, are significantly affected by high salt and low concentration. Therefore, removing salts and concentrating samples prior to these analyses are necessary in order to generate accurate and quantitative results.

Currently salt removal and concentration can be done by using molecular weight cut-off membrane spin columns. This type of spin column can adsorb proteins on the membrane leading to unpredicted and disproportional loss of sample. In addition, the need for high g-force and 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 gentle method for the desalting and concentration of biologics in a single easy step. The benefits of our method compared with current membrane-based spin columns:

- **No additional stress added to the proteins**
- **Single and rapid step: 4 min process vs. up to 20min process**
- **Up to 100% recovery**
- **Compatible with most downstream analyses**

Application: Concentration of Human Serum IgG Solutions Using the ConSep Column

Human serum IgG was dissolved in saline solution containing 0.9% (~154 mM) sodium chloride. IgG concentrations were prepared at 1.0 mg/ml, 0.5 mg/mL and 0.25 mg/mL. Concentration factors were calculated by comparing OD₂₈₀ before and after ConSep column treatment.

IgG Concentration Before mg/ml	IgG Concentration After mg/ml	Concentration Factor	Mass Recovery %
1.00	6.21	6.21	100.0
0.50	3.28	6.56	100.0
0.25	1.49	5.96	100.0

Application: Concentration and Desalting of a Human Serum IgG Solution - CE-SDS Analysis of Before and After Use of the ConSep Column

The 0.5 mg/ml Human serum IgG (above), dissolved in saline solution containing 0.9% sodium chloride, was analyzed using CE-SDS on a Beckman PA800. Before using the ConSep column, there was no IgG peak detected due to the low concentration and some salt suppression of the analysis. Following treatment of the sample with the ConSep column, the IgG peak is clearly visible (Fig. 1 below).

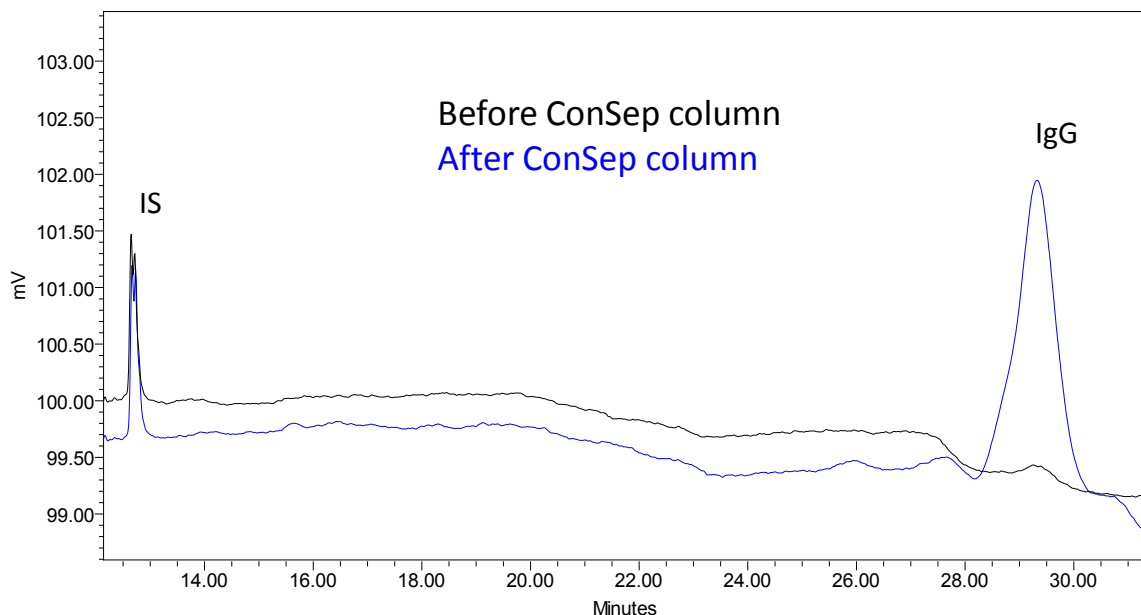


Fig. 1: Electropherograms of Human Serum IgG, 0.5mg/mL in PBS buffer, black: before using ConSep™; blue: after desalting and concentrating using ConSep column

Application: Concentration of a Therapeutic mAb After Using the ConSep Column

A hydrophobic therapeutic mAb was dissolved in PBS (~150 mM sodium chloride). The mAb concentrations were prepared at 1.0 mg/ml and 0.5 mg/mL. Concentration factors were calculated by comparing OD₂₈₀ before and after ConSep column treatment.

mAb Concentration Before mg/ml	mAb Concentration After mg/ml	Concentration Factor	Mass Recovery %
1.00	5.67	5.67	67%
0.50	2.03	4.07	65%