Monoclonal antibody CE-SDS purity analysis with the SCIEX low pH SDS sample buffer on the BioPhase 8800 system

BioPhase 8800 system

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Introduction

Monoclonal antibody (mAb) stability and purity analysis is an essential step in the development and manufacturing of this therapeutic modality or biosimilars. [1,2,3] As a result, biopharma scientists are faced with the analytical challenge of measuring the percent content composition of IgG molecules and impurities after production or during storage conditions. In this technical note, we describe the application of the high-quality and ready-to-use SCIEX low pH sample buffer as an additional biochemical tool to characterize the stability of thermally induced fragmentation of non-reduced IgG.[4] We also demonstrate the multiplexing capability the BioPhase 8800 system, a multi-capillary electrophoresis system for high-throughput analysis of protein therapeutics using the all-in-one SCIEX CE-SDS protein analysis kit. The SCIEX low pH sample buffer and the BioPhase 8800 system offer an additional analytical solution for mAb analysis during manufacturing, or for examining recommended IgG quality attributes for clinical applications or commercialization.

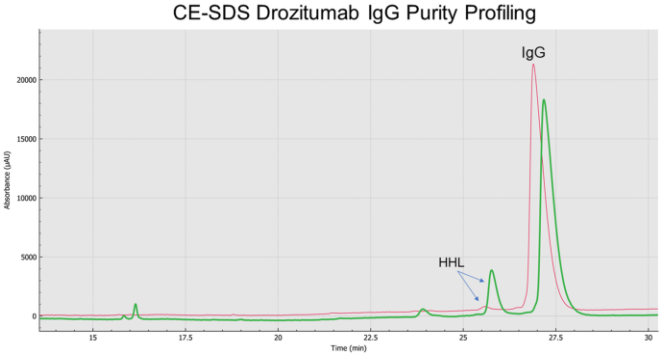

Figure 1. CE SDS purity analysis for drozitumab. Analysis using the SCIEX SDS-MW sample buffer (pH 9.0) is shown as the green trace, and using the SCIEX low pH sample buffer (pH 6.8) as the red trace. The SCIEX CE-SDS protein analysis kit (P/N 30085) contains high-quality and ready-to-use reagents and these sample buffers for mAb stability and purity analysis. As shown in this figure, drozitumab sample preparation for CE-SDS analysis showed a much lower impurity content using the SCIEX low pH sample buffer as indicated by the arrows pointing to the HHL products.

Figure 2. The SCIEX BioPhase 8800 system. It is a multi-capillary electrophoresis system that enables biopharma scientists to run multiple samples in parallel, accelerating the development and execution of sensitive, high-throughput analytical methods. The BioPhase 8800 system provides automated, quantitative, and repeatable capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) separations, designed to support the analytical needs in development and QA/QC, all in one system.

In more detail, we describe the process for the pH stabilizing effect on IgG molecules during mAb sample preparation using capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) and UV detection. For example, it has been shown that treating mAb samples with a basic pH sample buffer (9.0) or a slightly acidic pH sample buffer (6.8) and thermally induced fragmentation under alkylating and non-reducing conditions before CE-SDS analysis leads to a differential IgG purity profiling of the same mAb sample. In this case, it is done by monitoring IgG fragmentation or aggregated IgG species.

Key features

- Application of the SCIEX low pH SDS sample buffer for CE-SDS analysis of mAbs reduces artifacts
- In combination, the SCIEX low pH and the SDS-MW sample buffers for mAb sample preparation under alkylated and non-reducing conditions with the BioPhase 8800 system provide more accurate assessment of IgG stability and purity content
- The BioPhase 8800 system, including the analysis software, greatly reduce sample processing and analysis time, providing excellent CE-SDS repeatability and intermediate precision results based on the IgG profiling of three different biosimilars for drozitumab, infliximab, and adalimumab

Predominantly, showing that IgG molecules are more likely to dissociate or generate impurities in the presence of basic sample preparation conditions, in contrast to a cleaner IgG purity profile by applying a slightly acidic sample buffer during sample preparation, as a result of inhibiting the thiol-disulfide exchange reaction. [4]

To demonstrate this biochemical *pH* stabilizing effect on mAb samples in a high-throughput fashion reflecting manufacturing processes and QA/QC environments, we utilized commercially available biosimilars for drozitumab (anti-DR5), infliximab (anti-TNF alpha), and adalimumab (anti-TNF alpha). Subsequently, samples from these three different biosimilars were prepared for CE-SDS analysis using the BioPhase 8800 system in the presence of the SCIEX SDS-MW sample buffer (pH 9.0) or by using the SCIEX low pH SDS sample buffer (pH 6.8). In addition, to applying thermally induced fragmentation under alkylating and non-reducing conditions to both sets of sample preparation conditions. In summary, this technical note, provides an additional analytical tool to examine mAb stability and purity analysis.

Methods

Materials: The biosimilars for drozitumab, anti-DR5 human IgG1, *lambda* (P/N AB00740-10.0), infliximab, anti-TNF *alpha* human IgG1, *kappa* (P/N Ab00146-10.0), and adalimumab, anti-TNF *alpha* human IgG1, *kappa* (P/N Ab00718-10.0) monoclonal antibodies (mAbs) were obtained from Absolute Antibody, Redcar, Cleveland, United Kingdom. Iodoacetamide (P/N I1149) was purchased from Millipore-Sigma, St. Louis, Missouri. The CE-SDS protein analysis kit (P/N C30085, Figure 3) containing the SDS-MW sample buffer (pH 9.0), the low pH (pH 6.8) sample buffer, the Acidic and basic wash, CE-SDS gel, CE grade water, along with the BioPhase BFS (bare fused silica) capillary cartridge (8 x 30 cm) (P/N 5080121) were obtained from SCIEX, Framingham, Massachusetts.

Instrument and software: A BioPhase 8800 system (Figure 2, P/N 5083590) equipped with a UV detector was from SCIEX, Framingham, MA. Absorbance wavelength was set at 220 nm. Data acquisition and analysis were performed using BioPhase software v1.0 (SCIEX, Framingham, MA).

Sample preparation: First, a 500 µM solution of iodoacetamide (IAM) using distilled water was prepared and stored at 4ºC. Second, 5 µL of the prepared IAM solution was added to 50 µL of the mAb interest (1 mg/mL) that was mixed with either 45 µL of the SDS-MW sample buffer (pH 9.0) or with 45 µL of the low pH sample buffer (6.8). After a quick spin (10 seconds) samples were heated up at 70ºC for 5 minutes using a thermal cycler and allowed to cool down at room temperature for minimal of 10 minutes. To simulate intermediate precision, samples were prepared three different times over different days and each sample was injected into the BioPhase 8800 system four times.

BioPhase 8800 system methods and sequences*:* The BioPhase BFS capillary cartridge conditioning, separation, and shut down methods were created using the BioPhase software v1.0 as illustrated on Figure 4, panels A, B, and C, respectively. CE-SDS monoclonal antibody profiles were generated by running defined BioPhase 8800 system sequences depending on the experimental design. As an example, Figure 5 shows the sample and reagent plate layout for an 8-capillary run. This layout was sufficient to analyze 8 different samples, with each sample analyzed four times with a fully automated process.

Data analysis: Corrected Peak Area% was calculated on each run or mAb profile with the BioPhase software using the following parameters: drozitumab, 0.25% Positive Threshold, Suspend Integration from 0.0 to 12.5 minutes; infliximab, 0.08% Positive Threshold, Suspend Integration from 0.0 to 12.5 minutes; adalimumab, 0.2% Positive Threshold, Suspend Integration from 0.0 to 12.5 minutes. Peaks were merged as needed based on automatically detected peaks by the BioPhase software. Values were tabulated on a spreadsheet software program (Microsoft Excel) to calculate percent coefficient variation (%CV) and statistical significance using the student's t-test.

Figure 3. The CE-SDS protein analysis kit (P/N C30085) includes ready to use Acidic and basic wash, CE-SDS gel, CE grade water, SDS-MW sample buffer (pH 9.0), and the low pH SDS sample buffer (pH 6.8).

Panel A

Method Duration: 37.0 min Number of Actions: 7

Settings	Capillary Cartridge: 25.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA		Sample Storage: 25.0 °C, Wait Detector Type: UV, 220 nm, Wait Peak Width: 2 sec Data Rate: 4 Hz	
Rinse	Duration: 2.0 min 70.0 psi			Inlet: Basic Wash Outlet: Waste
Rinse	Duration: 8.0 min 20.0 psi			Inlet: Basic Wash Outlet: Waste
Rinse	Duration: 5.0 min 20.0 psi			Inlet: Acid Wash Outlet: Waste
Rinse	Duration: 2.0 min 20.0 psi			Inlet: Water Rinse Outlet: Waste
Rinse	Duration: 10.0 min 80.0 psi			Inlet: CE-SDS Gel Buffer Rinse Outlet: Waste
Separate	Duration: 10.0 min -15.0 kV, 20.0 psi, Both Ramp time: 5.0 min Autozero: 5.0 min			Inlet: CE-SDS Gel Buffer Sep Outlet: CE-SDS Gel Buffer Sep
Wait	Duration: 0.0 min			Inlet: Water Dip 1 Outlet: Water Dip

Panel B

Method Duration: 61.6 min Number of Actions: 12

Settings	Capillary Cartridge: 25.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 300 µA , Enabled		Sample Storage: 25.0 °C, Wait Detector Type: UV, 220 nm, Wait Peak Width: 2 sec Data Rate: 4 Hz	
Rinse	Duration: 2.0 min 80.0 psi			Inlet: Basic Wash Outlet: Waste
Rinse	Duration: 5.0 min 20.0 psi			Inlet: Basic Wash Outlet: Waste
Rinse	Duration: 5.0 min 20.0 psi			Inlet: Acid Wash Outlet: Waste
Rinse	Duration: 3.0 min 20.0 psi			Inlet: Water Rinse Outlet: Waste
Rinse	Duration: 10.0 min 80.0 psi			Inlet: CE-SDS Gel Buffer Rinse Outlet: Waste
Wait	Duration: 0.0 min			Inlet: Water Dip 1 Outlet: Water Dip
Rinse	Duration: 0.5 min 5.0 psi			Inlet: Water Rinse Outlet: Waste
Wait	Duration: 0.0 min			Inlet: Water Dip 2 Outlet: Water Dip
Inject	Duration: 65 sec 5.0 psi	Tray: Sample		Outlet: CE-SDS Gel Buffer Inj
Wait	Duration: 0.0 min			Inlet: Water Dip 3 Outlet: Water Dip
Separate	Duration: 35.0 min -15.0 kV, 20.0 psi, Both Ramp time: 1.0 min Autozero: 5.0 min			Inlet: CE-SDS Gel Buffer Sep Outlet: CE-SDS Gel Buffer Sep
Wait	Duration: 0.0 min			Inlet: Water Dip 1 Outlet: Water Dip

Panel C

Method Duration: 37.0 min Number of Actions: 7

Settings	Capillary Cartridge: 25.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA		Sample Storage: 25.0 °C, Wait Detector Type: UV, 220 nm, Wait Peak Width: 2 sec Data Rate: 4 Hz	
Rinse	Duration: 2.0 min 70.0 psi			Inlet: Basic Wash Outlet: Waste
Rinse	Duration: 8.0 min 20.0 psi			Inlet: Basic Wash Outlet: Waste
Rinse	Duration: 5.0 min 20.0 psi			Inlet: Acid Wash Outlet: Waste
Rinse	Duration: 2.0 min 20.0 psi			Inlet: Water Rinse Outlet: Waste
Rinse	Duration: 10.0 min 80.0 psi			Inlet: CE-SDS Gel Buffer Rinse Outlet: Waste
Wait	Duration: 0.0 min			Inlet: Water Dip 1 Outlet: Water Dip
UV Lamp	OFF			

Figure 4. Panels A, B, and C together show the cartridge conditioning, separation, and instrument shut down methods in a sequence for mAb CE-SDS profiling. This sequence was optimized to resolve mAbs treated with the SDS-MW sample buffer (pH 9.0) or the low pH SDS-sample buffer (pH 6.8). As shown in this sequence, an 8-sample run was completed in less than 2.5 hours.

Figure 5. Sample and reagent plates layout generated by the BioPhase software. This representative layout indicates that 8 samples (column 5, left side) were analyzed using the loaded CE-SDS proteins analysis kit reagents as shown on the reagent plates section (right side).

Results and discussion

CE-SDS drozitumab IgG purity and stability profiling

As shown on figure 1, and consistent with the biochemistry for the thiol-disulfide exchange equilibrium, a much greater IgG purity content was observed for non-reduced and alkylated drozitumab treated with the SCIEX low pH (6.8) SDS sample buffer (average IgG content 99.0%; *red trace*). The main impurity reflecting the Heavy-Heavy-Light Chain (HHL) product with an average impurity content of 0.9% (*arrows*). In contrast, CE-SDS sample preparation under basic conditions (pH 9.0) using the SCIEX SDS-MW sample buffer (*green trace*) highly favored IgG reduction as demonstrated by the appearance of IgG-related impurities, mainly the production of HHL products with an average content of 12.3%, resulting on an IgG average purity content of 82.3%. Furthermore, Table 1 summarizes the pH effect on the stability of drozitumab treated under slightly acidic or basic conditions. Mainly, greater fragmentation or artifacts were detected on drozitumab by using CE-SDS in the presence of a basic sample buffer.

Figure 6 shows the BioPhase software overlay for triplicates of drozitumab analyzed with the SCIEX SDS-MW sample buffer (pH 9.0) or the SCIEX low pH sample buffer (pH 6.8). In this case we demonstrated the excellent reproducibility of CE-SDS IgG purity profiling generated by using the sample preparation buffers, the reagents found in the CE-SDS protein analysis kit, and the BioPhase 8800 system. In more detail, and in addition to observing a decrease in HHL content with the low pH sample buffer, we also detected a decrease in other IgG components

like the heavy or light chains as seen in the area of the electropherogram labeled as other impurities. In summary, the combination of the SCIEX sample buffers (pH 9.0 or pH 6.8) can be used to provide a better assessment about the stability or purity of mAbs.

Analysis of infliximab with pH 9.0 and pH 6.8 sample buffers

As previously shown in previous studies, IgG alkylation during sample preparation using IAM is a key step to prevent aberrant product formation like IgG heavy molecular weight species due to thiol-disulfide exchange reactions. In this section, we present the CE-SDS infliximab purity profiling in the presence of the basic or slightly acidic sample buffers. Our results showed that infliximab sample preparation using thermally induced fragmentation under alkylated and non-reducing conditions with basic or slightly acidic buffers lead to detection of higher molecular weight (HMW) species as shown on figure 7 with a percent composition of less than 1% (Table 1).

However, closer analysis showed infliximab having an average HMW content of 0.5% in the presence of the pH 6.8 sample buffer and average HMW content of 0.4% using the pH 9.0 sample buffer (p-value of 0.014).

The HHL impurity for infliximab using this sample preparation procedure showed average content composition of 0.8% using the pH 6.8 sample buffer, in contrast to an HHL impurity of 1.4% in the presence of the pH 9.0 sample buffer ($p = 1.86 \times 10^{-9}$).

Lastly, the intact IgG purity under slightly acidic conditions was greater than with using a basic sample buffer during sample preparation. In this case, an average IgG purity content of 97.7% and 96.9% ($p = 3.80 \times 10^{-5}$), respectively.

Increased adalimumab IgG purity detected with SCIEX low pH sample buffer

BioPhase 8800 system analysis of adalimumab IgG purity showed that sample preparation also played a role on IgG purity profiling. For example, the overlay or stacking of the electropherograms for non-reduced and alkylated adalimumab samples in the presence of the basic or slightly acidic SCIEX sample buffers for CE-SDS analysis, as shown on figure 8 and Table 1, demonstrated a much bigger HHL peak (*green trace*) for samples treated with the basic buffer in contrast to the adalimumab treated with the low pH sample buffer (*red trace*).

Table 1. mAbs CE-SDS purity intermediate precision on the BioPhase 8800 system. CE-SDS intermediate precision (%CV) for drozitumab, infliximab, and adalimumab purity content was performed with the BioPhase 8800 system. BioPhase software calculated the Corrected Area% to quantify the main peak (IgG) and related impurities for drozitumab, infliximab, and adalimumab. One arm of this study consisted of the biosimilars treated with the SDS-MW sample buffer (pH 9.0), the second arm treated drozitumab, infliximab, and adalimumab with the low pH sample buffer (pH 6.8). Collectively, HHL and HWM impurities accounted for the majority of mAb impurities. In general, HHL impurities or artifacts were minimized under alkylated and non-reduced conditions using the SCIEX low pH sample buffer (pH 6.8).*HMW: High Molecular Weight; HHL: Heavy-Heavy-Light Chain

Antibody		% Total fragments	% Main peak purity	% Total HMW* species	%HHL*
Drozitumab in sample buffer pH 9.0	Average	17.7	*82.3*	*0.0*	*12.3*
	%CV	8.5	*1.8*	*n/a*	*7.7*
Drozitumab in sample buffer pH 6.8	Average	*1.0*	*99.0*	*0.0*	*0.9*
	%CV	*11.3*	*0.1*	*n/a*	*6.7*
Infliximab in sample buffer pH 9.0	Average	*3.1*	*96.9*	*0.4*	*1.4*
	%CV	*10.7*	*0.3*	*28.8*	*1.8*
Infliximab in sample buffer pH 6.8	Average	*2.3*	*97.7*	*0.5*	*0.8*
	%CV	*2.7*	*0.1*	*13.2*	*6.1*
Adalimumab in sample buffer pH 9.0	Average	*2.0*	*98.0*	*0.0*	*1.0*
	%CV	*6.5*	*0.1*	*n/a*	*5.5*
Adalimumab in sample buffer pH 6.8	Average	*0.6*	*99.4*	*0.0*	*0.3*
	%CV	*13.6*	*0.1*	*n/a*	*9.7*

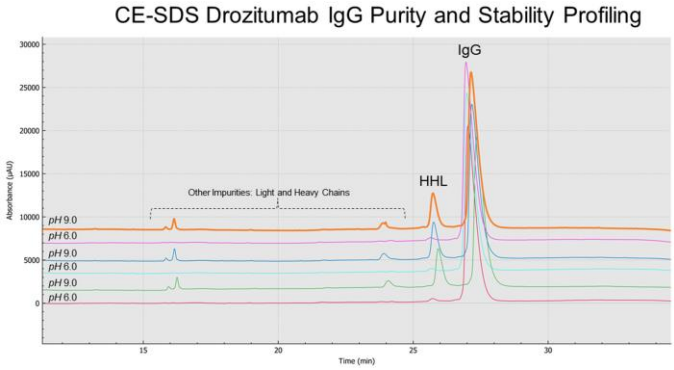

Figure 6. Drozitumab CE-SDS IgG purity and stability analysis. Simultaneous analysis of six samples, three replicates by using the SCIEX SDS-MW sample buffer (pH 9.0), and three other replicates with the low pH sample buffer (pH 6.8) were performed using the BioPhase 8800 system and the protein analysis kit (P/N C30085). Less artifacts or impurities were detected using the SCIEX low pH sample buffer.

Specifically, adalimumab's IgG (% main peak purity) average content was 99.4% in the presence of the SCIEX low pH sample buffer. The average IgG content for adalimumab treated with the SCIEX SDS-MW sample buffer was 98.0%, making for a statistically significant difference of $p = 1.88 \times 10^{-13}$ for the IgG purity under these two sample preparation conditions. No HMW species were detected for adalimumab treated with the basic or slightly acidic SCIEX sample buffers.

The average adalimumab HHL content composition for the acidic and basic sample preparation conditions were 0.3% and 1.0%, respectively ($p = 4.15 \times 10^{-13}$).

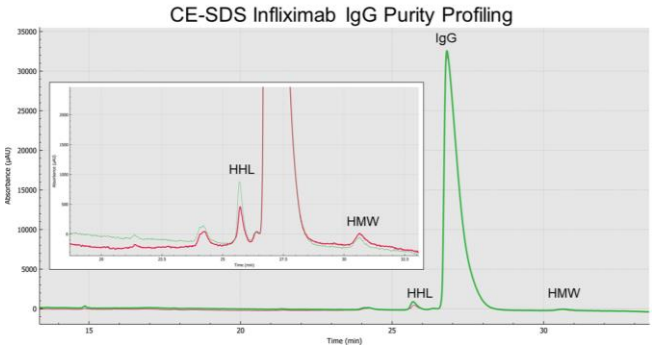

Figure 7. Infliximab treated with the SCIEX SDS-MW sample buffer, pH 9.0 (green trace) and the SCIEX low pH SDS sample buffer, pH 6.8 (red trace). BioPhase software quantified an average IgG purity of 97.7% using the low pH sample buffer. The average IgG purity under basic conditions was 96.9%. The HHL impurity was higher using the basic pH sample buffer (1.4% vs. 0.8%). HMW species were detected at less than 1% for both conditions.

BioPhase 8800 system performance on IgG purity profiling

To support the characterization of IgG purity in development and QA/QC, we performed an intermediate precision CE-SDS study on drozitumab, infliximab, and adalimumab using the BioPhase 8800 system.

Table 2. Infliximab's repeatability on the BioPhase 8800 system. Simultaneous sample processing for mAb profiling using alkylated and non-reduced conditions with the low pH (6.8) or high pH (9.0) sample buffers. These series of injections, n=4, for each buffer condition support the application of the BioPhase 8800 system for mAb development studies and QA/QC criteria. * T-test based on three independent sample preparations, each sample preparation injected three times for analysis. ** HMW: High Molecular Weight; HHL: Heavy-Heavy-Light Chain

Antibody	Replicate injection	% Total fragments	% Main peak purity	% HMW* species	%HHL* Peak
Infliximab in sample buffer pH 9.0	1	3.6	*96.4*	*0.3*	*1.4*
	2	*3.4*	*96.6*	*0.3*	*1.4*
	3	*3.6*	*96.4*	*0.3*	*1.4*
	4	*3.7*	*96.3*	*0.4*	*1.5*
Infliximab in sample buffer pH 6.8	1	*2.3*	*97.7*	*0.4*	*0.9*
	2	*2.2*	*97.8*	*0.4*	*0.8*
	3	*2.3*	*97.8*	*0.5*	*0.8*
	4	*3.0*	*97.0*	*0.5*	*0.8*
% CV pH *buffer 9.0*		*3.6*	*0.1*	*8.9*	*3.8*
% CV pH *buffer 6.8*		*15.7*	*0.4*	*5.3*	*5.4*
Main Peak %, p-value** (pH 9.0 vs pH 6.8)		*3.80 x 10 -5*			
HMW Species %, p-value** (pH 9.0 vs pH 6.8)		*1.43 x 10 -2*			
HHL %, p-value** (pH 9.0 vs pH 6.8)		1.86 x 10 -9			

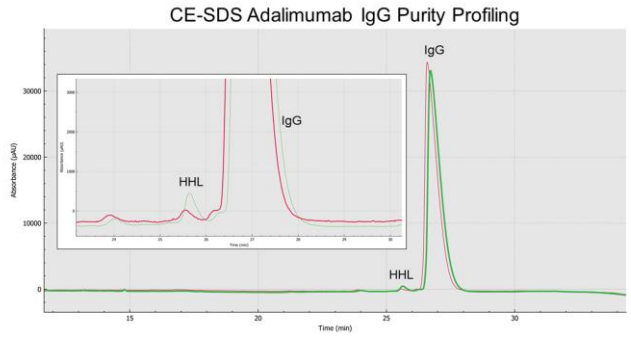

Figure 8. **Adalimumab treated with the SCIEX SDS-MW sample buffer**, pH 9.0 (*green trace*) and the SCIEX low pH SDS sample buffer, pH 6.8 (*red trace*). BioPhase software quantified an average IgG purity of 99.4% using the low pH sample buffer. The average IgG purity under basic conditions was 98.0%. The HHL impurity was higher using the basic pH sample buffer (1.0% *vs.* 0.3%). No HMW species were detected.

Following alkylated and non-reduced mAb sample preparation conditions for thiol-disulfide exchange reactions using the slightly acidic or basic sample preparation buffers as noted in the Methods section for CE-SDS analysis this intermediate precision study consisted of three different sample preparations over three different days, with each sample preparation analyzed four times (*n=4*). In summary, Table 1 illustrates the average percent composition for the main peak or IgG and impurities such as HHL, HWM, and other fragmentation species for drozitumab, infliximab, and adalimumab in the presence of the basic or slightly acidic pH buffers. The least variation (*%CV*) on IgG purity content determination consistently across drozitumab, infliximab, and adalimumab by CE-SDS using the BioPhase 8800 system and the CE-SDS protein analysis kit, was by preparing the mAb sample with SCIEX low pH SDS sample buffers. Note that % Total Fragments represents all artifacts or impurities prior to the IgG peak and the HWM species. All the %CV values for the % Total Fragments across the different mAbs were calculated below a 15% cut off. However, we recommend that analytical labs follow the most current acceptance criteria guidelines for impurity assay development or qualification.

Overall, the different %CV values shown on Table 1 support the analytical capability of the BioPhase 8800 system for development and QA/QC applications. In more detail, Table 2 indicates the individual values for infliximab's %Total Fragments, %Main Peak, %HMW species, and %HHL Peak calculated by the BioPhase 8800 software. This representative series of injections based on the mAb sample preparation using the basic or slightly acidic buffer demonstrate the high reproducibility of impurities profiling by applying the SCIEX CE-SDS protein kit and the simultaneously analyses performed by the BioPhase 8800 system.

Conclusions

- UV CE-SDS IgG purity content determination using the BioPhase 8800 system demonstrated excellent robustness for development and QA/QC characterization
- High-throughput processing using a fully automated platform characterized up to 72 injections or mAb profiles with minimal BioPhase 8800 system interaction. Saving hours or days of sample processing time compared to other single channel CE-SDS systems.
- IgG purity profiling can differ when the sample preparation is performed with a basic pH buffer (pH 9.0) or a slightly acidic pH buffer (pH 6.8)
- Parallel mAb sample preparation using the SCIEX SDS-MW sample buffer (pH 9.0) and the low pH SDS sample buffer (pH 6.8) can provide a better assessment for IgG stability and purity profiling

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