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Novel Interface for High-Throughput Analysis of Biotherapeutics by Electrospray Mass Spectrometry

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Abstract

With the rapid rise of therapeutic antibodies and antibody-drug conjugates, significant investments have been made in developing workflows that utilize mass spectrometry to detect these intact molecules, the large fragments generated by their selective digestion, and the peptides generated by traditional proteomics workflows. The resultant data is used to gain insight into a wide range of parameters, including primary sequence, disulfide bonding, glycosylation patterns, biotransformation, and more. However, many of the technologies utilized to couple these workflows to mass spectrometers have significant limitations that force nonoptimal modifications to upstream sample preparation steps, limit the throughput of high-volume workflows, and prevent the harmonization of diverse experiments onto a single hardware platform. Here, we describe a new analytical platform that enables direct and high-throughput coupling to electrospray ionization mass spectrometry. The SampleStream platform is compatible with both native and denaturing electrospray, operates with a throughput of up to 15 s/sample, provides extensive concentration of dilute samples, and affords similar sensitivity to comparable liquid chromatographic methods.

Graphical Abstract

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Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.analchem.9b04826>.

Figure S-1: schematic diagram of fluid/valve configuration in the SampleStream system; Figure S-2: calibration curve for the SampleStream device using trastuzumab; Figure S-3: spectrum resulting from 10 ng load of trastuzumab analyzed with the SampleStream Platform; Figure S-4: elution flow rate analysis; Figure S-5: direct comparison of IgG1 (150 kDa) processed by SampleStream with a single focusing event and the full method; Figure S-6: direct comparison of liquid chromatography and the SampleStream device for several biotherapeutics; Figure S-7: analysis of biotherapeutics in native mode; Figure S-8: deconvoluted spectra of the antibody-based drug trastuzumab, Fab-IgG1, and noncovalent anti-Ox40 hexamer; Figure S-9: spectra generated by liquid chromatography and the SampleStream device for THIOMAB/XTEN Conjugate; Figure S-10: direct comparison of trastuzumab and XTEN conjugate through chromatography and SampleStream; and Figure S-11: timing diagram showing overlapping mode of operations (PDF)

Notes

The authors declare the following competing financial interest(s): PDC, JJD, and NLK are owners of Integrated Protein Technologies, Inc., the manufacturer of the SampleStream platform.

The undisputed importance of biopharmaceuticals has energized the fields of intact protein analysis and top-down mass spectrometry. In these analytical approaches, mass spectrometers are utilized to study proteins without enzymatic digestion, yielding a holistic view of proteins and their associated proteoforms.¹ Now, with over 60 monoclonal antibody drugs approved by the Food and Drug Administration (FDA),² both pharmaceutical companies and academicians have rapidly developed targeted workflows for antibodies and antibody drug conjugates that provide information on composition, disulfide bonding, glycosylation, degradation, biotransformation, and more.^{3–18} The combination of these measurements affords the level of precision required by regulatory agencies worldwide to confidently assess the safety and efficacy of these drugs.

While the terminal detector for these methods is almost exclusively a mass spectrometer, upstream sample preparation and separation approaches vary widely based on application. Traditional reversed phase chromatography is used extensively for intact measurements of the products of deamidation, oxidation, isomerization, clipping, and more.^{19,20} Other forms of chromatography, including size exclusion and ion exchange, have been utilized to operate in a native modality that preserves noncovalent interactions.^{21–23} Hydrophobic interaction chromatography (HIC) is also routinely used to evaluate the drug-to-antibody ratio of antibody-drug conjugates. High-throughput systems for direct infusion as well as solid phase extraction see use in applications where the number of samples to be analyzed demands it. While this list cannot be inclusive of all approaches utilized, it does demonstrate the wide range of interfaces used to introduce samples to mass spectrometers.

The commonality in all of these interfaces is the ease of integration and automation they afford. In fact, in many applications, chromatography systems are utilized in a trap-and-elute modality where samples are introduced to a column then rapidly eluted following on-column buffer exchange. These methods provide sample cleanup and concentration while producing very little separation of analytes, serving mainly as convenient means for sample introduction to a mass spectrometer. Unfortunately, utilizing chromatography as a simple automation interface is inefficient for a variety of reasons, including the cost and complexity of the equipment, the limitations placed on solvent composition (both for the sample and mobile phase), and the inability to harmonize various methods onto a single platform.

Other approaches, including high-throughput solid-phase extraction (SPE) (e.g., Agilent's RapidFire), direct infusion (e.g., Advion's TriVersa), and droplet-based approaches²⁴ are capable of yielding very high throughput analyses using electrospray but can be limited to

samples that are already relatively free of salts and other contaminants. Further, these options require the purchase of specialized equipment even beyond the liquid chromatography (LC) systems as well as the development of additional expertise.

Herein, we present a new platform technology that addresses these limitations. On the basis of a fluidic device that functions at low pressure (<30 bar), the platform accepts a wide range of matrices and solvents and can process samples at a rate of 15 s/sample. When compared to competing LC-based methods, the prototype device analyzes therapeutic antibodies and larger constructs with equivalent sensitivity and spectral quality. The platform maintains this unprecedented level of data quality and throughput in both native and denaturing modes.

EXPERIMENTAL SECTION

SampleStream Platform Hardware.

The SampleStream device was constructed utilizing 3 Tecan Cavro XLP syringe pumps fitted with 250 μ L syringes and high-pressure 9-port distribution valves. One or two PEEK channels containing a sintered stainless-steel frit and a 10 kDa PL or PLC molecular weight cutoff (MWCO) membrane (Millipore) was/were connected to a 10-port injection valve mounted on the rail of a PAL3 robot (CTC Analytics), as well as a second 2-position 6- or 10-port valve (Idex-HS) mounted to the SampleStream module. All plumbing consists of PEEK tubing connected with standard finger-tight fittings. The system also integrates pressure transducers (DJ Instruments) that monitor the inlet of each channel and a dual output temperature controller (Omega Engineering) that powers a heater tape and monitors temperature with a K-type thermocouple. Embedded electronics consist of a G120E development board (GHI Electronics) running the .NET microframework (v.4.3) and a home-built analog board containing two input and two output contact closure circuits. A rendering of the system is provided in Figure 1.

SampleStream Platform Software.

The embedded electronics contain firmware that controls the pumps and valves via RS232, sets the output contact closures with a digital I/O pin, monitors input contact closures and pressure readbacks with analog input pins, and communicates to the PC via TCP/IP. IPT was given access to prerelease software from CTC Analytics that enables the development of a “plugin”. The plugin enables the activities of the SampleStream module to be exposed directly to the PAL 3’s scripting language. The scripts generated in this language were then run through Thermo Fisher Scientific’s Xcalibur software utilizing the Thermo TriPlus autosampler driver which is compatible with the PAL3 robot, or the device and robot were controlled directly by CTC Analytics’ DirectAPI through a custom program written in C#.

System Operation.

A schematic diagram of the fluid/valve configuration for the single channel system is shown in Figure S-1. The system was operated using a PAL 3 script as described above. Briefly, the flow control valve was set to the focusing position to load and focus the sample in the system. The syringe pumps (P1 and P2) were then filled with the buffer solution, and the sample was loaded into an injection valve with a sample loop by the autosampler (PAL 3,

CTC Analytics, Zwingen, Switzerland). Subsequently, the injected sample was washed with the buffer solution within the device during the focusing step. After washing, the pumps were refilled with a system liquid, which was delivered to the device to buffer-exchange and preconcentrate the sample during the following focusing step. After focusing, the flow control valve was automatically switched to the elution position, and then the resulting sample was directly eluted by a syringe pump (P3) to the mass spectrometer.

Liquid Chromatography.

A Dionex Ultimate 3000 nano-LC system was fitted with a 2.1 mm PLRP-S column (Agilent) heated to 60 °C. Mobile phase A was 0.1% formic acid and mobile phase B was acetonitrile (ACN) and 0.1% formic acid. The gradient was 0 min 20% B; 2 min 20% B; 4.75 min 65% B; 5 min 80% B; 5.5 min 15% B; 5.75 85% B; 6 min 15% B; 6.25 85% B; and 6.5 min 15% B. The flow rate was 100 $\mu\text{L}/\text{min}$. Samples were loaded in 5 μL using the microliter pickup injection strategy.

Samples.

Variants of Anti-Ox40 including Fab-IgG1 and RGY hexamer were generated as described previously.²⁵ The variants of Anti-Ox40 and the RGY hexamer were supplied in 20 mM L-histidine and 150 mM NaCl (pH 5.5), and PBS, respectively. Trastuzumab was provided by Genentech with excipients as a powder containing 440 mg Trastuzumab, 9.9 mg L-histidine HCl, 6.4 mg L-histidine, 400 mg a,a-trehalose dihydrate, and 1.8 mg polysorbate 20 USP.

Mass Spectrometric Analysis.

For denatured antibody analysis, samples were analyzed by a Q Exactive (QE) Plus mass spectrometer (Thermo Fisher Scientific) coupled to an Ion Max source (Thermo Fisher Scientific) fitted with a HESI II probe with a flow rate of 25–200 $\mu\text{L}/\text{min}$. Mass spectra were acquired at a resolution setting of 17,500 with a scan range from m/z 1,600 to 6,000 and settings including a spray voltage of 3.7 kV, a sheath-gas flow rate of 7, an in-source collision-induced-dissociation (SID) value of 20 eV, and a capillary temperature of 320 °C. For native antibody analysis, samples were analyzed by a QE UHMR mass spectrometer (Thermo Fisher Scientific) coupled to an Ion Max source fitted with a HESI II probe with a flow rate of 100 $\mu\text{L}/\text{min}$. Mass spectra were acquired at a resolution setting of 12,500 with a scan range from m/z 2,500 to 15,000 and settings including a spray voltage of 4.3 kV, a sheath-gas flow rate of 20, and a capillary temperature of 275 °C. Ion transfer optics throughout the instrument and in-source trapping fragmentation voltage were tuned for every sample specifically. For the noncovalent antibody complex, 125 °C of probe heater temperature was applied. Mass spectra were viewed in Thermo Xcalibur Qual Browser software (Thermo Fisher Scientific).

Denaturing SampleStream Method.

Samples were loaded in system liquid (30% ACN with 0.2% acetic acid) where the concentration of ACN was set to maximize matrix removal efficiency while maintaining stable electrospray,²⁶ and acetic acid was added to adjust the pH for denaturing MS analysis. Samples were focused in the channel with 187.5 μL of 20 mM ammonium acetate followed

by 562.5 μL of system liquid. Samples were then eluted from the channel at 100 $\mu\text{L}/\text{min}$ with system liquid. The total method duration for SampleStream was 1 min 45 s. All samples were analyzed using the same tune file and mass spectrometer. This method was used for all analyses unless otherwise noted.

Native SampleStream Method.

Equimolar injections of each sample, starting with 500 ng of trastuzumab, were made. Injected protein was focused in 187.5 μL of 50 mM ammonium acetate then eluted in the same. The flow rate for elution was 100 $\mu\text{L}/\text{min}$, yielding a total method time of 50 s.

Metrics of Analytical Performance.

The quantitative performance of the device was assessed by loading varying amounts of trastuzumab into the SampleStream module in 100 μL in triplicate starting with the lowest concentration and analyzed with the denaturing method described above. Between each injection, an injection of water was recorded. Detector response for both analytical and carryover injections was estimated by applying a mass range filter to the data selecting all peaks from m/z 1,600 to 4,000. The area of the resultant peak in the extracted ion chromatogram was determined by software integration using Qual Browser (Thermo Fisher Scientific).

For the characterization of elution volume, 500 ng of trastuzumab was loaded in 10 μL then eluted with different flow rates. Extracted ion chromatograms were used to determine the peak volume at the full width half-maximum of the peak.

Concentration enrichment was assessed by loading 500 ng of trastuzumab in various volumes. The area of extracted ion chromatograms were determined by software integration.

Comparison of Liquid Chromatography to the SampleStream Module.

Each of the samples described previously was analyzed via LC, utilizing the method described above, and with the SampleStream module. The instrument and tune files were identical between the two data series.

High-Throughput Operation.

A SampleStream module with two channels was integrated with a dual head PAL 3 robot (CTC Analytics). The system was operated in an overlapping mode with one channel undergoing focusing while the other was eluting. Samples were focused with 187.5 μL of system liquid. Samples were eluted in system liquid at 300 $\mu\text{L}/\text{min}$. Total analysis time was 30 s/channel.

BioPharma Finder.

Mass spectra were deconvoluted using the Intact Protein Analysis module of BioPharma Finder 3.0. The ReSpect deconvolution algorithm was used starting from default settings, and mass ranges and charge state ranges were adjusted as necessary for each analyte. Charge state distributions were identified with a deconvolution mass tolerance of 50 and 30 ppm for the RGY hexamer and large hydrodynamic radii species, respectively.

RESULTS AND DISCUSSION

The SampleStream module is a new fluidic device that enables simple and high-throughput coupling of a variety of workflows directly to electrospray mass spectrometry. The platform consists of 3 syringe pumps, an injection valve, a flow control valve, and the fluidic channel, all integrated with a PAL 3 robot (CTC Analytics). Within the fluidic channel, a volume is created by the geometry of a machined PEEK body over a MWCO membrane that is supported by a sintered metal frit (Figure 2A).

The channel is operated with one of two fluidic circuits. In one circuit, samples are delivered to the channel and undergo fluidic focusing (Figure 2A), similar to the process utilized in asymmetric flow field flow fractionation (AF4). During this phase, molecules retained by the action of the MWCO membrane migrate to the point in the channel where the incoming axial flows cancel (focusing). Concurrently, the molecules are driven down toward the membrane (relaxation), forming a tight band that occupies a fraction of the overall channel volume. We confirmed that the band is predictably formed at a distance down the channel axis that corresponded to the ratio of inflows (data not shown), which is similar to previous reports in AF4 literature.²⁷ Once focused, the flows to the channel are modified to enable elution of the samples in a concentrated band (Figure 2B) reminiscent of traditional chromatographic methods. While simple in concept, the SampleStream device provides impressive analytical performance for a range of applications.

The selection of the membrane for this study is based on two major factors. The first factor is retention of the desired protein. In our experience, the best recovery of the protein was achieved when utilizing a cutoff that is 6 $\times$ less than the molecular weight of the analyte of interest, which is consistent with the documentation for these membranes provided by the supplier. The second consideration is that of the system. Decreasing MWCO filters generate increased back pressure at a set flow rate. This trend is exponential in nature. Thus, the optimal selection for a membrane is the highest MWCO that achieves the 6 $\times$ difference between MWCO and the analyte MW. As, in this study, we focused on the analysis of biotherapeutic antibodies with molecular weights of more than 150 kDa, a 10 kDa MWCO membrane was utilized for further experiments.

Metrics of Analytical Performance.

Trastuzumab with excipient was used exclusively as a representative sample in a battery of experiments utilized to quantify traditional metrics of performance. The quantitative response of the device was assessed with repeated injections at various mass loading. The device produced a linear detector response from 10 to 500 ng (Figure S-2) (r -squared: 0.998). Even at 10 ng loaded (~66 fmol), trastuzumab was detected with $S:N > 10$ (Figure S-3). Further, blank injections performed between each analytical injection reveal carryover of <5% at all but the lowest loading amounts (Figure 3A). Likely, this is due to the method of calculation which simply uses a peak area measurement after application of a mass range filter as described in the methods. Utilizing this method, any chemical/electronic noise present will account for an increasing proportion of signal measured as the absolute amount of carryover decreases, leading to overestimation of carryover at low loading amounts.

In addition to these more common metrics, the SampleStream module has two other unique characteristics. In Figure 3B, equimolar injections of trastuzumab were made with different loading volumes in triplicate. The average integrated peak area across all of these injections was $5.39 \times 10^9 \pm 2.83\%$, indicating that the resultant data was highly consistent, regardless of the loading amount. This consistency is also verified by the consistency in elution volume at FWHM, plotted as a line in Figure 3B. For each loading volume, dilution factors were calculated by comparing the loading volume to the elution volume at FWHM. The bars in Figure 3B indicate that the system actually results in concentration (dilution factor <1) of samples loaded in volumes above the elution volume of the device. Therefore, the more dilute the starting sample, the higher the concentration enrichment becomes following focusing (Figure 3C).

Unlike chromatography in which plate height is affected by the linear velocity of the mobile phase during elution, the elution volume for species eluted from the SampleStream device is not affected by elution flow rate, except at the lowest flow rate (Figure S-4). Further, equimolar loading of samples in various volumes are also eluted in a consistent elution volume. To summarize, the elution volume of the system is insensitive to loading amount and, to a large extent, elution flow rate, enabling precise prediction of the dilution/concentration factor under a wide range of experimental conditions.

Comparison to Conventional Methods.

The simplest mode of operation for the SampleStream device mimics the trap-and-elute style measurements performed in high-throughput targeted workflows. Biopharma utilizes this workflow extensively for characterization of therapeutic antibodies and related molecules. Antibody-based samples were analyzed using established chromatographic methods and again with the SampleStream device, all on the same mass spectrometer with the same tune file. The elution flow rates were matched to produce the most accurate comparison possible. Each sample, ranging in molecular weight from ~150 kDa to ~250 kDa was loaded in equimolar amounts, starting with 500 ng of trastuzumab (3.3 pmol), and analyzed with both methods. Figure 4 displays the resultant mass spectra for analysis of a Fab-IgG1 250 kDa species. Examination of the spectra reveals equivalent spectral quality. Figure S-5 compares spectra acquired after different focusing volumes in the SampleStream device, showing improved desalting with increasing focusing time. The charge envelope from the SampleStream device is slightly shifted to lower charge. The other species assayed produced similar data, presented in Figure S-6. The shift in charge state distribution indicates one of two possibilities. First, denaturation is not an instantaneous process. After being focused in a native buffer, the unfolding of the proteins after being reintroduced to a denaturing buffer may be incomplete on the time scale of the experiment. Second, the frit that supports the membrane may be retaining low concentrations of ammonium acetate, causing a shift to higher pH during elution.

The lower panel of Figure 4 is the overlay of the total ion chromatogram from the two analyses with the peaks aligned in time. Here again, the performance of the SampleStream device is equivalent to the optimized LC methods that are currently in use. It should be noted, however, that in these experiments, while loading between the two techniques was

equimolar, loading volumes differed. Samples were injected into the SampleStream device in 100 μL , while the loading volume for the corresponding LC experiments was only 5 μL . This result reiterates the ability of the device to provide quantitative concentration of highly dilute samples. Further, the method times for the SampleStream device and LC differed by a factor of 2.5 in this experiment, yielding a significantly higher throughput when utilizing the SampleStream module.

The recovery efficiency from SampleStream is 88.0% when analyzing 500 ng of IgG1, dissolved in 20 mM His and 150 mM NaCl (pH 5.5), in triplicate. However, the coefficient of variation for recovery of all the antibodies used to compare with LC averaged 1.7%. While all of the species analyzed are related as they are all antibody constructs, the consistency in recovery for all of them suggests that, at least for this class of compounds, the performance is independent of the analyte.

Native Mass Spectrometry.

Another unique aspect of the SampleStream device is its insensitivity to loading buffer composition. In contrast to LC, analytes are not bound to a stationary phase, so no consideration must be given to partitioning. This feature enables the device to rapidly switch between native and denaturing conditions, with the only change to methods being the system liquid.

The same samples utilized for the denaturing work were subsequently reanalyzed utilizing a Q Exactive UHMR under native conditions. The methods remained identical with the exception of utilizing 50 mM ammonium acetate at pH 7.0 as the system liquid. Figure 5A,B shows the resultant spectra of the trastuzumab (150 kDa) and Fab-IgG1 (250 kDa) species. In this case, each piece of data required 80 s to acquire, inclusive of autosampler time. Further, the spectra were generated using equimolar loading, starting with 500 ng of trastuzumab (3.3 pmol). Native spectra of the other molecules analyzed are presented in Figure S-7.

These data represent a clear advantage over current methods for covalently or genetically linked antibody complexes. Subsequent experiments were done to assess performance for noncovalent species. A noncovalent hexameric version of anti-Ox40 (produced using Fc variants E345R/E430G/S440Y)²⁵ was analyzed with the same method for the 150 kDa and 250 kDa species, with the only change being the modification of source and ion transfer settings on the UHMR. Even at 100 $\mu\text{L}/\text{min}$, with flow rates much higher than characteristic of the static nanospray approaches commonly used for native analysis, the hexamer was detected intact (Figure 5C) with a quality sufficient for deconvolution with BioPharma Finder (Figure S-8).

Accepting both native and denaturing buffers encompasses a wider range of solvent compatibility than the individual LC techniques compared to herein. In addition, several different solvents were tested, including 20% dioxane, 20% DMSO, 20% isopropyl alcohol, 30% ACN, and 100 mM ammonium acetate, due to the extensive chemical compatibility of PEEK and the regenerated cellulose membrane. The resulting data was unremarkable and

these solvents produced similar data to the solvents described in the methods (data not shown).

Atypical Antibody Formats.

As already demonstrated, the unique characteristics of the SampleStream device make it particularly well-suited to molecules with increasing molecular weight. In addition, emerging drug platforms have other interesting characteristics that are also amenable to analysis with the SampleStream device. A final comparison between LC and the SampleStream device was made using a THIOMABTM antibody/XTEN conjugate with a molecular weight of ~300 kDa but with a hydrodynamic radius corresponding to a spherical molecule with MW > 2 MDa. Following deconvolution of the two spectra (Figure S-9), only the data produced by the SampleStream device resulted in the detection of the intact species (Figure S-10). Here, the SampleStream device uniquely enables the analysis of a family of therapeutics that simply cannot be analyzed with existing methods.

Ultra-High-Throughput Mass Spectrometry.

While much of the preliminary data contained herein was collected using a single channel system with a single head PAL 3 robot, an upgraded version was constructed to push the limits of throughput. The updated platform contains two channels on a dual-head PAL 3. The use of 3 syringe pumps, 2 for focusing and 1 for elution, enables the development of overlapping methods. In this approach, one channel is undergoing focusing while the other, containing a previously focused sample, is undergoing elution (diagrammed in Figure S-11).

Combined with dual-head PAL 3, this layout enables ultrahigh-throughput operation. Figure 6 demonstrates repeated analyses of trastuzumab in the denaturing mode, producing a real throughput of 30 s/sample. However, each channel was operating with a total method time of 30 s, which theoretically would produce a throughput of 15 s/sample. For this experiment, limitations in the PAL 3 rail layout created approximately 15 s of dead time for each injection where the SampleStream module was inactive. Therefore, improvements to the rail layout that eliminates this overhead will result in realized throughput of 15 s/sample. This level of throughput for antibody analysis is unprecedented via LC or SPE techniques let alone in a native modality.

Not only does the system produce a stunning throughput in both native and denatured mode, the coefficient of variation (CV) for peak area of the data from each of the two channels individually averaged 3%. When combined, this number rose to nearly 8%. The prototype device used for this experiment was not calibrated to equalize the volumes of the flow paths for the two channels and each channel was hand machined. We anticipate the performance of individual channels to align more closely as production and plumbing are regularized. Further, blank injections immediately following the analytical injections reinforce the extremely low carryover reported previously of <5%.

To minimize the possibility of cross contamination, which could be a potential concern for high throughput analysis of different samples, the SampleStream system was setup to automatically flush the injection port with wash solution after sample injection. Additionally, the PAL 3 robot LCMS tool utilizes an internal PET sample loop to prevent

sample from ever entering the syringe. This loop is also flushed after every injection with both a weak and strong wash. These steps mitigate cross contamination caused by the autosampler itself. Additionally, given that sample carry-over between runs within the channel is below 5% with the ultralow protein binding membranes used in this study, the channel itself is expected to perform similarly to traditional chromatography. In cases where further reduction in carryover is required, a blank injection can be performed between analytical injections.

CONCLUSIONS

The SampleStream device represents a new and dramatically simplified platform for coupling upstream workflows to mass spectrometry through electrospray ionization. With several unique characteristics, the device improves upon existing workflows and, moreover, enables many that are not currently possible. Its insensitivity to solvent composition enables denaturing and native workflows to coexist while a defined elution volume enables quantitative recovery from large volumes of highly dilute samples. Combined with a linear response over a wide range of mass loading, this technology represents a potent platform that has applications in biopharma and beyond.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
A photorealistic rendering of the SampleStream Platform.

Figure 2.

Depiction of the two modes of operation for the fluidic channel where sample concentration and vigorous buffer exchange occur above a membrane. (A) During focusing, a flow stream enters both top ports and molecules retained by the membrane migrate to the equilibrium point while buffer and small molecules exit through the membrane and frit. (B) During elution, flow is delivered from under the membrane while one top port is blocked, forcing molecules retained by the membrane to exit the downstream port and out of the fluidic channel for downstream analysis.

Figure 3.

(A) Measured carryover from the blank runs performed immediately after analytical injections. (B) The dilution factor and elution volume calculated after performing equimolar loading of the antibody trastuzumab in different loading volumes. (C) Plot illustrates that the signal remains similar at a wide range of concentrations based on equal amount loading. Error bars represent standard error of the mean for three technical replicates for each data point.

Figure 4.

(Top) Comparison of a 250 kDa Fab-IgG1 antibody analyzed with liquid chromatography (left) and the SampleStream (SS) device (right). (Bottom) The corresponding elution profiles for the LC and SampleStream peaks aligned in time with calculated peak areas.

Figure 5.

(A) A Native mass spectrum of the antibody-based drug trastuzumab (150 kDa). (B) Native mass spectrum of Fab-IgG1. (C) Native mass spectrum of RGY hexamer (880 kDa).

Figure 6.

Extracted ion chromatogram of high-throughput injections alternating with blanks labeled with peak area CV, cycle time, and average carryover. The number of repeats of the run for carryover estimation is 20 ($n = 20$). Inset shows typical mass spectrum from a sample injection.