Application of inline variable pathlength technology for rapid determination of dynamic binding capacity in downstream process development of biopharmaceuticals

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Abstract

Determination of dynamic binding capacity (DBC) for capture purification chromatographic step is usually the first experiment to be performed during downstream process development of biopharmaceuticals. In this work, we investigated the application of inline variable pathlength technology using FlowVPE for rapid determination of DBC on affinity resins for protein capture and proved its comparability with offline titer methods. This work also demonstrated that variable pathlength technology for DBC determination can be successfully applied to different classes of monoclonal antibodies and fusion proteins. This enabled rapid screening of affinity resins and optimization of the capture chromatography step. Hence, use of inline variable pathlength technology eliminated the dependency on offline titer data, traditionally used for DBC determination and accelerated overall process development timelines with less cost.

KEYWORDS

affinity, biopharmaceuticals, capture, downstream process development, dynamic binding capacity, FlowVPE, resin screening, variable pathlength

1 | INTRODUCTION

An ever-increasing market for biomolecule-based therapies is driving biopharma companies to accelerate process development activities to remain competitive in today's pharmaceutical industry. The reduction in drug to market time can be accomplished by utilizing real-time process analytical tools and techniques, which provide an overall gain in efficiency.1–3 KBI Biopharma, Inc. has explored one such avenue by implementation of a FlowVPE system for rapid determination of dynamic binding capacity (DBC) for the capture purification step for different classes of monoclonal antibodies and fusion proteins. This has helped in realizing substantial time and cost savings associated with downstream process development of biomolecules.

The capture purification step is usually one of the first investigations performed while developing a downstream process for biopharmaceuticals like monoclonal antibodies.4,5 Affinity chromatographic media, such as Protein A resins are the most popular choice for this step allowing for selective capture of the target protein from clarified cell culture harvest. Affinity chromatography has firmly established itself in industrial manufacturing of biologics due to high yield and high-purity streams generated in a single step. This simplifies process development downstream of capture, and as such, it has become difficult to replace with an equivalent alternative.6 However, the cost of affinity resins is a major challenge during large scale GMP manufacturing. Protein A resins, which are the most commonly used affinity resins, typically cost $10,000–$20,000/liter and are 3–9 times more expensive than non-
affinity resins. Resin cost for a large Protein A column (>1 m diameter) can exceed $1 million. Depending on the column size, cost of affinity resins can account for 30%-50% of the total downstream raw material cost during an antibody drug manufacturing campaign. It was reported that costs associated with use of Protein A affinity resins in biopharmaceutical manufacturing is determined by resin binding capacity, cycling numbers and resin pricing. As a result, DBC determination studies are often the first experiments to be performed for resin selection for the capture step. The goal is maximum utilization of the binding capacity of the affinity resin to keep the column size small and achieve an economical process.

There are a variety of affinity resins in the market with different ligand density, ligand design, and base matrix design. The most preferred affinity ligand, namely Protein A, has been attached to a large variety of different base matrices such as cross-linked agarose, surface modified porous glass, coated polystyrene, hydrogel filled into a ceramic shell, and other materials based on organic polymers. Although resin binding capacity is largely governed by the biomolecule structure and ligand density, each resin also has its own flow characteristics which may be an impactful factor in resin selection and decisions regarding the operating conditions. For the same molecule, resin binding capacity can vary as large as 2-folds on different resins. It is thereby common, during development of capture purification step, to screen at least two affinity resins and one or two residence times. This in turn necessitates determination of DBC 2-4 times per molecule and is a significant contributing factor to the process development time for the capture step.

DBC is defined as the amount of a target protein bound to the resin under determined flow conditions before significant breakthrough of unbound protein occurs. DBC is determined by loading a sample of the desired protein at target flow conditions and monitoring the fluoro through the resin. breakthrough point occurs when the effluent concentration reaches 10% of the starting concentration of the target protein in the feed. This in turn necessitates determination of DBC 2-4 times per molecule and is a significant contributing factor to the process development time for the capture step.

In theory, a real time breakthrough curve can be generated by monitoring the pure protein concentration in column flow through with the FPLC built-in UV detector at 280 nm. However, when using clarified harvest as feed and due to the limitation of the built-in UV detector, generation of a real time breakthrough curve for capture resins using these inline fixed pathlength UV detectors do not produce accurate results in most cases. The inline UV detectors that are part of an AKTA Avant FPLC system have a fixed pathlength of 2 mm and are rated as ±2% linearity at 0-2000 mAU (Cytiva AKTA Avant Data file). The nontarget proteins in the flow through of the affinity resin during the load phase can often generate >2000 mAU of base level UV absorbance, oversaturating the inline FPLC UV detector. It has been shown that due to the high UV absorbance of the media and impurities, there was a minimal difference in UV280 nm signal at a 2 mm pathlength during column loading phase. The observations indicated that base UV level absorbance of the clarified harvest can greatly affect the change in UV280 nm signal and mask the target protein breakthrough at a 2 mm pathlength, making it difficult to precisely calculate the DBC.

In order to overcome the limitations of built-in UV detectors, efforts have been made to predict loadings on Protein A columns in real time using optical methods including UV/Vis absorption spectroscopy, near infrared spectroscopy, and single-wavelength delta UV measurements. In one method, antibody concentration in the column effluent is monitored by multiwavelength spectroscopy together with multivariate data analysis to monitor the target protein breakthrough. However, the multivariate partial least-squares (PLS) regression models require calibration prior to breakthrough curve assessment to account for the variations of cell culture medium components and target molecule concentrations, which limits its application in industry. The
complex data analysis and mathematical modeling also complicate the overall operation.

An NIR-based PAT approach has also been proposed for controlling loading on continuous chromatography capture columns using a near infrared spectroscopy flow cell incorporated in the flow path.\textsuperscript{20} Similar to UV/Vis multiwavelength spectroscopy, calibration and PLS models are needed to convert NIR spectra data to protein concentration data, which is not efficient for routine industry operations.

Another approach, known as the single-wavelength delta UV method, utilizes the UV 280 absorbance between the load material and column effluent to calculate column breakthrough. To overcome the UV detector saturation challenges, fixed UV cell path length ranging from 0.05 to 1 mm was used in the study to achieve a robust difference in delta UV.\textsuperscript{17} Though the method provides inline effluent concentration, application of this method may be limited due to the complexity of dual UV detectors setup. The UV cells path length also needs to be adjusted for each clarified harvest feed stream depending on the harvest base level UV absorbance and target molecule titer. Additionally, the method is only suitable for clarified harvest with titers higher than 3 g/L.\textsuperscript{17}

Recently, inline variable pathlength UV spectroscopy system became commercially available and has been assessed for real time monitoring of protein concentration in mixtures. It was reported that integrating a single FlowVPE system downstream of a chromatography column can monitor the separation of lysozyme from cytochrome C in real-time.\textsuperscript{21} In addition to this, variable pathlength UV spectroscopy has been reported to enable determination of antibody titer in harvest by calculating the difference between the absorbances generated by target protein depleted clarified harvest and starting load material. The titers so obtained were comparable to the traditional Protein A high-performance liquid chromatography (HPLC) titers.\textsuperscript{19} These results led us to the hypothesis that it is feasible to utilize a single FlowVPE system to monitor protein breakthrough during affinity capture step using clarified harvest as the feed stream. Therefore, independent of the earlier studies, KBI Biopharma has thoroughly studied the inline variable pathlength technology by the application of FlowVPE and has built a rapid affinity resin DBC assessment platform. Our data demonstrated that this method enables real time generation of breakthrough curves with high-frequency data collection and does not involve lengthy and cumbersome offline titer data analysis; thus, leading to convenient determination of DBC using considerably less resources and time.

2 | EQUIPMENT, MATERIALS, AND METHODS

2.1 | FlowVPE

FlowVPE is an inline variable pathlength spectrophotometer developed by C Technologies (Repligen) that is adaptable to a wide range of protein sample concentrations (0.1–250 g/L according to vendor manual) and modalities.\textsuperscript{1,22} The software allows the instrument to auto range its pathlengths from 0.005 to 8 mm so that the absorbance is within linear range of Beer’s law for the sample being measured. It utilizes a slope-based technique that takes the linear regression of 5+ datapoints (Absorbance vs. Path Length) to generate a slope value of the sample.\textsuperscript{23} Concentration is then calculated by dividing the slope value by the target molecule’s extinction coefficient (EC).\textsuperscript{23} This instrument is based on the same variable pathlength technology utilized by SoloVPE (C Technologies, Repligen) that is widely used for convenient and accurate protein concentration determination.\textsuperscript{23,24} FlowVPE can take inline measurements with collections time as quick as 5 seconds per cycle to monitor the protein concentration of the samples passing through its flow cell.

During DBC assessment, the FlowVPE flow cell was integrated into an AKTA Avant flow path downstream of a column outlet to facilitate inline and real time measurement of effluent concentration at 280 nm. The data acquisition using FlowVPE method was manually started immediately upon start of the sample application phase. The column volume and system holdup volume were accounted for during loading and DBC calculations. The connection leading to FlowVPE was a small diameter tubing with minimum required length. This combined with the holdup volume of 0.75 ml for a 3 mm Flow cell, led to negligible peak broadening effect when using a 20 ml column. Figure 1 shows the flow path configuration.

2.2 | Chromatography procedures

For each molecule studied, prior to affinity capture, harvest clarification was performed by the upstream process development team at KBI Biopharma. Clarification included depth and sterile filtration, and in some cases centrifugation. Protein A affinity resins MabSelect Sure LX (Cytiva Life Sciences, Marlborough, MA, USA), MabSelect PrismA

![Flow path configuration for DBC assessment using FlowVPE. DBC, dynamic binding capacity](image-url)
system. For breakthrough curve determination, only the concentration versus time data is utilized.

The first step of the data analysis process is determining the baseline concentration of the target protein depleted harvest forming the flow through, before breakthrough occurs (theoretically this accounts for background level UV absorbance attributed to all components in the mixture except the target protein). In brief, mean value of the protein concentration of the flow through fraction (baseline absorbance) before product breakthrough is calculated and then subtracted from all the effluent concentration values. This difference corresponds to target protein concentration in the flow through stream and turns into positive values only when the product starts appearing in the flow through as protein breakthrough. The target protein concentration is then divided by the feed titer to yield the percentage breakthrough.

This data so obtained can be further processed to generate the final break through curve as percentage product breakthrough as a function of column loading factor. The column-loading factor (X-axis in Figure 2C) is calculated from flow rate, time, harvest titer, and column volume.

\[
\text{BT} = \frac{\text{Target Protein Concentration in effluent fraction}}{\text{Concentration of target protein in Clarified Harvest}} \times 100.
\]

### 2.4 Breakthrough curve generation by offline titer method

Approximately 20 fixed volume effluent fractions were collected during the load phase, the offline titer was determined using a ProA-HPLC or BioHT titer method. The breakthrough percentage for the target protein was calculated using the Equation (2) and plotted against column load factor to generate the breakthrough curve.

The FlowVPE has two data acquisition and analysis modes—KF (Fixed Slope Kinetics) and KQ (Quick Slope Kinetics). For the DBC determination work performed at KBI Biopharma, the KQ mode at 280 nm wavelength has been used extensively since it is more suitable for gradual concentration changes occurring during DBC determination. One slope data point per 15 s is the default data collection frequency for most of the studies. For projects with lower harvest titer, the FlowVPE instrument was programmed to acquire data points per 8 s to reduce noise levels, resulting in smoother breakthrough curves. Every 8–15 s time intervals, FlowVPE measures multiple absorbance readings at various path lengths with absorbance threshold at 1.0 AU. A slope value is calculated using this absorbance data to determine the sample protein concentration. The data acquired from the Flow VPE software is in the form of three variables, namely, effluent concentration, \( R^2 \) and slope values as a function of time. \( R^2 \) values typically depict the accuracy of measurement and can be inspected to ensure the health of the system. For breakthrough curve determination, only the concentration versus time data is utilized.

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\[
\text{Mass Loaded} = \frac{\text{Flow Rate} \times \text{Time} \times \text{Harvest Titer}}{\text{Column Volume}}.
\]
3 | RESULTS AND DISCUSSIONS

3.1 | Inline protein concentration monitoring by FlowVPE method

The setup described in the Equipment, Materials, and Methods section was used to monitor the breakthrough of a monoclonal antibody (Figure 2). Figure 2a,b show comparison of chromatograms generated using the built-in AKTA UV detector and the FlowVPE at 280 nm. UV traces between approximately 75 and 340 min correspond to the loading and wash phases. The peak at approximately 340 minutes represents the product elution. The flow through UV signal from the AKTA system was between 2000 and 4000 mAU, which exceeded the inline UV cell linearity and made it difficult to accurately determine where the 10% breakthrough occurs. The elution peak UV absorbance also saturated the UV detector. On the other hand, the same chromatogram captured by FlowVPE reported protein concentration of the effluent stream and elution peak in real time as a function of time. Note the scale on the Flow VPE chromatogram for Y-axis has concentration units because the EC of the target protein can directly be applied during measurement to convert absorbance into concentration. In Figure 2b, the protein concentration trace between approximately 75 and 340 min does not reflect the actual protein concentration but the combined absorbance due to the mixture of harvest and target protein. However, after subtracting the baseline absorbance, inline protein concentration data for the target protein (Figure 2b) can be easily converted to a breakthrough curve (Figure 2C) following the procedure outlined in the methods sections.

3.2 | Comparability between HPLC titer and FlowVPE method

In order to understand the feasibility of replacing HPLC based titer methods with FlowVPE for breakthrough curve generation, efforts were first directed to establish comparability between results derived from the two methods. Figure 3 shows the breakthrough curves obtained for capture purification of a bispecific antibody and a Fc-fusion protein on a Protein A affinity resin using both flow through fraction ProA-HPLC titer and inline FlowVPE concentration data. It is evident that both offline titer and FlowVPE data breakthrough curves showed similar trend of protein breakthrough profiles (Figure 3). For Bispecific mAb1, offline titer analysis of fractions yielded a DBC of 48 g/Lr whereas the FlowVPE data resulted in a DBC value of 46 g/Lr. As can be seen, there is a close correlation between DBC data obtained from both these methods for Bispecific mAb 1 and this data set represents less than 5% difference between the DBC values determined by the two methods (Figure 3a and Table 1).

To gain more insight into the comparability between the offline titer and FlowVPE methods, breakthrough curves were generated side by side for multiple molecules and various affinity media. Table 1 summarizes the DBC data obtained using both methods and it was observed that differences in the calculated DBC values were less than...
10% for each case. The mean DBC value difference is 1.8 g/L between offline titer method and FlowVPE method. A paired t-test using JMP statistical software suggests that the two sets of data are not statistically different (95% confidence interval). It is hypothesized that DBC values from both methods would converge if more offline titer data points were collected.

One important aspect of breakthrough curve determination using FlowVPE worth emphasizing is the greater resolution it provides in DBC calculation. Although the two breakthrough curves are similar in Figure 3a, Figure 3b shows the limitation of the offline titer method during DBC assessment. Due to the steep rise in breakthrough curve, the first data point for product breakthrough was detected at 13% breakthrough by offline titer method and the 10% DBC value had to be interpolated from the discontinuous breakthrough curve. FlowVPE allows for high-frequency data collection during inline measurement of effluent concentration resulting in a smooth breakthrough curve. FlowVPE can determine protein concentration every 15 s, which is equivalent to data point for every 1.25 or 0.75 ml of column effluent when flow rate used is 300 or 180 cm/h, respectively. In contrast, traditional offline analytical titer method measurements are limited by number of effluent samples collected and results in a discrete breakthrough curve (Figure 3). This is due to the fact that offline titer methods rely on concentration measurement of individual effluent fractions and the resolution of the DBC curves generated from traditional titer method can depend greatly on the number and volume of these fractions. Calculations show that to achieve the same resolution as FlowVPE, offline titer analysis will need to be performed on 373 fractions, with a volume of 1.25 ml each, assuming a harvest titer of 3.0 g/L. When using ProA-HPLC method to determine the offline product titer in fractions, less than 48 samples are reasonable for testing to control the time and labor costs. Although selected samples can be submitted based on the visual inspection of the UV trace, there is a high probability of missing the effluent concentration data at the exact instance the 10% breakthrough occurs, especially if the harvest titer is high causing a steep breakthrough curve slope. This will lead to loss of critical information due to generation of discrete breakthrough curves and result in imprecise calculation of DBC. Therefore, the FlowVPE provides a sufficient data set enabling high-resolution breakthrough curve for determination of the DBC value.

Also as shown in Table 1, FlowVPE has an applicability over a wide range of molecule types and harvest titer values. It can be used effectively for DBC determination for molecules ranging from monoclonal antibodies to fusion proteins and bispecific antibodies. It was suspected that the FlowVPE inline UV absorption method would present challenges for precise determination of target protein concentration if the titer is lower than 1 g/L, because of the high baseline nontarget protein level. However, as the data shown in Table 1 indicates, breakthrough curve generation was achieved for a Fc-fusion protein having a low-harvest titer of 0.6 g/L and resulted in DBC value being less than 10% different between the two methods. This data demonstrated that FlowVPE is a suitable instrument for DBC determination of low-titer molecules. Together, this data confirmed that FlowVPE can be used as a viable alternative to traditional methods for DBC determinations.

**FIGURE 3** Breakthrough curves for a bispecific mAb and a Fc-fusion protein generated by ProA-HPLC offline titer and inline FlowVPE Data. FlowVPE determines DBC by multiple data points. HPLC data was processed to use linear interpolation between the two surrounding data points to predict DBC. DBC, dynamic binding capacity; HPLC, high-performance liquid chromatography

**TABLE 1** Comparison of DBC values obtained for capture purification step from offline titer method and FlowVPE data

| Molecule type  | Resin type  | Clarified harvest titer (g/L) | DBC by offline titer (g/Lr) | DBC by FlowVPE (g/Lr) | % difference |
|----------------|-------------|-------------------------------|-----------------------------|-----------------------|--------------|
| Fc-fusion 1    | Protein A   | 0.6                           | 40                          | 43                    | 8            |
| Bispecific mAb 1 | Protein A   | 2.4                           | 46                          | 48                    | 4            |
| Bispecific mAb 2 | Protein A   | 2.7                           | 29                          | 31                    | 7            |
| Bispecific mAb 3 | Protein A   | 0.9                           | 52                          | 55                    | 6            |
| mAb 1          | Protein A   | 6.1                           | 62                          | 61                    | 2            |
3.3 Application of FlowVPE based DBC assessment

The data described in the above sections demonstrate that breakthrough curve determination using the FlowVPE instrument can be applied to many important categories of biopharmaceuticals—mAbs, fusion proteins, and bispecific antibodies, to replace time consuming offline titer method. One major aspect of the real time DBC determination using inline variable pathlength technology is that this method provides an important tool for rapid screening of different resins.

During these studies, we noticed that the DBC value is impacted by both resin type and molecule structure (Figure 4, Table 2). Figure 4a represents a two resin screening study for bispecific mAb 1, which shows that DBC on ProA Resin A is almost 10 g/Lr higher than Resin B. In contrast, for mAb3, both Resin A and Resin B have comparable DBC that is slightly higher than Resin C (Figure 4b). The DBC values obtained for these two studies and other molecules are tabulated in Table 2. It is clear that mAb 5, bispecific mAb 1, and bispecific mAb 2 showed noticeably higher DBC on one of the assessed resins using the same processing conditions. It will be wiser to choose the resin with higher DBC to attain better process efficiency. On the other hand, although two resins may result in similar

### Table 2

| Molecule      | Clarified harvest titer (g/L) | DBC on ProA resin A (g/Lr) | DBC on ProA resin B (g/Lr) | DBC on ProA resin C (g/Lr) |
|---------------|------------------------------|---------------------------|---------------------------|---------------------------|
| mAb 2         | 2.7                          | 65                        | 65                        |                           |
| mAb 3         | 2.8                          | 58                        | 58                        | 56                        |
| mAb 4         | 3.9                          | 58                        | 53                        |                           |
| mAb 5         | 2.8                          | 64                        | 55                        |                           |
| Bispecific mAb 1 | 2.4                  | 64                        | 56                        |                           |
| Bispecific mAb 2 | 2.7                  | 64                        | 45                        |                           |
| Bispecific mAb 4 | 1.2                  | 62                        | 65                        | 77                        |

FIGURE 4  Breakthrough curves generated by FlowVPE during Protein A resin screening for a bispecific mAb (a) and a mAb (b)

FIGURE 5  Breakthrough curves generated by FlowVPE during residence time screening on a Protein A column for bispecific mAb 1 (a) and bispecific mAb 3 (b)
binding capacity for the target molecule (mAb 2,3,4, and bispecific mAb 4 in Table 2), there may be a significant cost difference between resins which can drive the resin selection decision in favor of one particular resin over another.

In some cases, other than resin screening, FlowVPE is also used for DBC comparison at different flow rates to select column loading residence time for optimum performance. As shown in Figure 5, the longer residence favors higher DBC. However, longer residence time can lead to lower loading flow rate that can slow down the unit operation. Hence, determination of DBC values on one resin at different flow rates can help the manufacturing team to model facility fit aimed at balancing the loading factor and operation time.

In all these resin selection cases, speed of DBC determination can be a critical factor for resin decision making and eventually leading to significant cost savings during large scale manufacturing. Such assessments at a desired speed cannot be efficiently executed without the help of the FlowVPE. This technique has been used for successfully determining DBC for over 20 molecules and helped with accelerating process development timelines.

Due to the ease and speed of operation, there are many potential applications for implementation of FlowVPE. Comparing resins of different lots based on the nature of the breakthrough curves to determine lot-to-lot variability is an important application and can be extended to resin lifetime and resin reuse studies as well. In addition to regular IgG and Fc-Fusion protein development, all these developed and underdeveloped applications can also be extended to recombinant enzymes.

### 3.4 Cost analysis of FlowVPE versus offline HPLC titer method

Based on the data shown in previous sections, the biggest benefit of employing FlowVPE for DBC determination is elimination of analytical testing cost and overall process development timeline improvement, similar to those offered by SoloVPE.24 The inline FlowVPE-based method was able to generate near real-time results for DBC determination with data analysis completed in less than 1 h. In contrast, traditional HPLC method takes an average of 2 weeks to report fully reviewed data if data is generated and analyzed by a separate analytical team. This slow data turn around delays a resin selection decision considerably and has a negative impact on the overall timeline. One of the factors causing these delays is attributed to the fact that analytical methods need to be developed before supporting these studies. In a contract research organization setting with tight timelines and parallel development of multiple molecules, this acts as a bottle neck. Offline titer methods also require manual sampling of the fractions, hand off to the analytical personnel and elaborate buffer preparations causing delays. The DBC measurements may not be performed by downstream process development personnel and there may be a constant dependency on the analytical team. Transitioning to inline technique for DBC determination enables a single person to accomplish this task and eliminates reliance on external factors to accelerate the overall process development. The material and labor cost of the DBC assessment analytical testing is worth approximately $15,000 per project. Although FlowVPE system costs ~$90,000, the break-even point can be reached through development of ~10–15 molecules, which is not atypical for a contract development and manufacturing organization (CDMO) like KBI Biopharma that supports greater than 10 development projects a year on average. This analytical cost estimation does not consider the HPLC instrument capital cost, or any additional cost related to sample retesting caused by testing errors. The risk due to possible errors occurring during analytical testing can be mitigated by using the FlowVPE method since it is largely operator independent. Additionally, the HPLC analytical columns have to be replaced routinely, the frequency of which is a function of the nature of the clarified harvest and varies widely from molecule to molecule. In contrast, fibrettes and window kits are the only consumables of FlowVPE that need replacement as needed, which is as infrequently as once per year, if the instrument is used correctly. Besides the obvious financial cost saving, near real-time DBC data is a critical step to accelerate downstream process development to meet KBI Biopharma’s aggressive process development timelines.

The inline variable pathlength technology can be extended to non-affinity based capture for proteins like enzymes where additional advantages can be realized. Analytical method development for titer determination for nonstandard molecules consumes more resources than traditional mAbs and implementing real time measurements can provide important timesavings. Since, the FlowVPE flow cell is compatible for usage in GMP settings, this technology can potentially also be used as a PAT tool for controlling loadings on Protein A columns for continuous processing.

### 4 Conclusions

This work provides a verified protocol and comprehensive analysis for rapidly determining DBC for various molecules on capture chromatography resins using the FlowVPE spectrophotometer based on inline variable pathlength technology. The data show that the resolution of this method is better than the traditional HPLC method. Using FlowVPE for DBC assessment during a capture purification step saves at least 2 weeks of analytical testing time and cost and eliminates downstream process development idle time. Thus, for capture chromatography step breakthrough curve generation, the Flow VPE is an ideal choice to replace the current offline HPLC titer method to realize considerable time and resources savings.

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### Author Contributions

Rashmi Bhangale: Conceptualization (lead); formal analysis (lead); investigation (supporting); methodology (lead); visualization (supporting); writing – original draft (lead); writing – review and
editing (lead). Rui Ye: Formal analysis (supporting); investigation (supporting); validation (lead); visualization (supporting); writing – review and editing (supporting). Thomas Lindsey: Formal analysis (supporting); investigation (lead); visualization (supporting); writing – review and editing (supporting). Leslie Wolfe: Resources (lead); supervision (lead); writing – review and editing (supporting).

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DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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