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A reversed phase HPLC method for the quantification of HIV gp145 glycoprotein levels from cell culture supernatants

José A. González-Feliciano [a,f], Coral M. Capó-Vélez [a,f], Pearl Akamine [a,f], Manuel Delgado-Vélez [a,f], Ruth Almodóvar [b,f], Javier Rivera [b,f], Ignacio Pino [b,f], Gloriner Morell [b,f], Daniel Eichinger [b,f], José H. Rivera [b,f], José A. Lasalde-Dominicci [a,c,d,f], Abel Baerga-Ortiz [a,e,f,*]

[a] *Molecular Sciences Research Center, University of Puerto Rico, San Juan, PR 00926, USA*
[b] *CDI Laboratories, 12 W Méndez Vigo Ave. Mayagüez, PR 00680, USA*
[c] *Department of Biology, University of Puerto Rico, Río Piedras Campus, San Juan, PR 00926, USA*
[d] *Department of Chemistry, University of Puerto Rico, Río Piedras Campus, San Juan, PR 00925, USA*
[e] *Department of Biochemistry, University of Puerto Rico, Medical Sciences Campus, San Juan, PR 00936-5067, USA*
[f] *Clinical Bioreagent Center, University of Puerto Rico, San Juan, PR, 00926, USA*

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ABSTRACT

A reversed phase high performance liquid chromatography (RP-HPLC) method was developed for the quantitative determination of recombinant HIV-1 gp145 produced in CHO-K1 cells, as measured directly from culture supernatants. Samples were diluted in 50% D-PBS and 50% PowerCHO-2 (PC2) spent medium, and resolved on a Zorbax 300SB-C8 Rapid Resolution (2.1 × 50 mm, 3.5 μm) column, fitted with a C8 guard column (Zorbax 300SB-C8, 2.1 × 12.5 mm, 5 μm), using 0.1% TFA and 2% *n*-propanol in LC-MS water as mobile phase A and 0.1% TFA, 70% isopropanol, and 20% acetonitrile in LC-MS water as mobile phase B. The column temperature was 80 °C, the flow rate was 0.4 mL/min and the absorbance was monitored at 280 nm. The procedures and capabilities of the method were evaluated against the criteria for linearity, limit of detection (LOD), accuracy, repeatability, and robustness as defined by the International Conference on Harmonization (ICH) 2005 Q2(R1) guidelines. Two different variants of the HIV-1 envelope protein (Env), CO6980v0c22 gp145 and SF162 gp140, were analyzed and their retention times were found to be different. The method showed good linearity (R^2 = 0.9996), a lower LOD of 2.4 μg/mL, and an average recovery of 101%. The analysis includes measurements of accuracy, inter-user precision, and robustness. Overall, we present a RP-HPLC method that could be applied for the quantitation of cell culture titers for this and other variants of HIV Env following ICH guidelines.

1. Introduction

The envelope (Env) glycoprotein that spans the membrane of the human immunodeficiency virus (HIV) and mediates viral infection has been a common template for the design of numerous vaccine candidates [1–9]. In HIV-infected cells, Env is naturally made as a membrane-spanning gp160 glycoprotein that is cleaved into two glycoprotein fragments: a trimeric gp41 and its monomeric binding partner gp120. The monomeric gp120 was used as the boost immunogen in the RV144 clinical trial in Thailand; the only vaccine trial that has so far resulted in significant HIV protection with an efficacy level of 31.2% [10]. The gp120 monomer is still under evaluation in clinical trials in Thailand and South Africa [11–13]. Although some of the early results obtained with the gp120 monomer as a boost immunogen were promising, the development of a globally protective immunogen will require proteins that elicit a more durable and targeted response.

The search for Env-based vaccines of higher efficacy and breadth has led some researchers to look beyond monomeric gp120. More recent immunogen designs have aimed to preserve the trimeric structure of the native Env spike, by including portions of trimer-forming gp41 covalently linked to the otherwise monomeric gp120 [6–8,14]. In this new category of trimeric Env immunogens there are uncleaved trimers (due to a mutated cleavage site), native trimers (gp120:gp41 complexes held together by an engineered disulfide bond), and several genetically fused

* Corresponding author at: Department of Biochemistry, University of Puerto Rico, Medical Sciences Campus, PO Box 365067, San Juan, PR 00936-5067, USA.
E-mail address: abel.baerga@upr.edu (A. Baerga-Ortiz).

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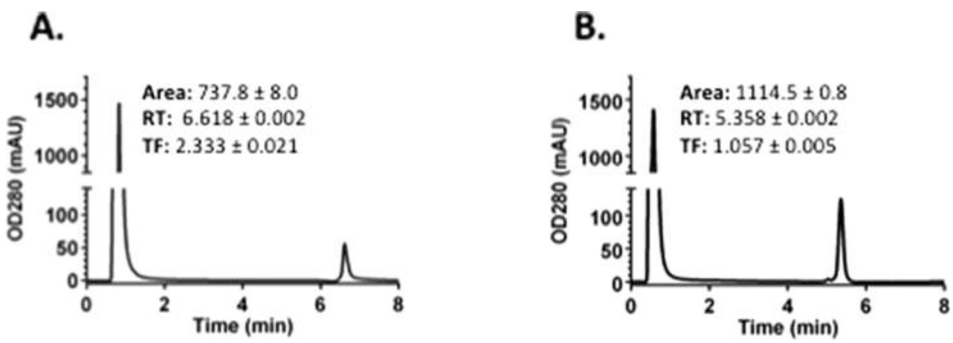

Fig. 1. RP-HPLC method optimization. Representative chromatograms obtained from three injections of the HIV-1 CO6980v0c22 gp145 RM (100 µg/mL) into a Zorbax 300SB-C8 rapid resolution RP-HPLC Column (2.1 × 50 mm, 3.5 µm) mounted on an Agilent BioInert Infinity II 1260 System with different sample preparation and elution gradient schemes. RT, retention time and TF, tail factor. (A) Condition A. The sample was mixed as follows: 40% D-PBS, 40% Mobile phase A, and 20% CHO-K1 spent medium. The elution gradient used was (time/%B): 0/30, 3.5/30, 4/65, 4.5/65, 5.5/75, 6.0/75, 6.5/80, 7.0/95, 12/95 and 12.5/35, with a post time of 5 min. (B) Condition B. The sample was prepared as follows: 50% D-PBS and 50% CHO-K1 spent medium. The elution gradient used was (time/%B): 0/30, 2/30, 3.5/65, 4/70, 5/75, 6.5/95, 9.5/95, and 10/30, with a post time of 5 min.

constructs (gp120:gp41 held together by a flexible peptide linker). Many of these new trimeric Env constructs, like their monomeric predecessors, are made in mammalian cell expression systems such as CHO and HEK-293, both of which are known to decorate the protein with a native-like glycosylation pattern [15–20]. However, the typical yields of Env-based proteins made in these tried and tested cellular hosts is about 10–100 times lower than the yields for other glycoproteins of pharmaceutical relevance, posing both a challenge and an opportunity for the optimization of upstream processes [18].

To facilitate the optimization of cultivation parameters during upstream and downstream process development, it is important to monitor Env production directly in the supernatants of cultured cells. To date, the most widely used quantitation method for secreted soluble Env immunogens in the culture media is the ELISA method [15–17,21]. Although ELISA is sensitive and inexpensive, comparative studies have shown that the reproducibility of the ELISA method is low, probably due to the variability of the antibodies used [22].

In this work, we describe the development of a reversed phase HPLC assay for the quantitative detection of the CO6980v0c22 gp145 trimer directly from a culture of CHO-K1 cells that were stably transfected to express the immunogen [7]. We present the results obtained as part of the development, optimization, and qualification of the RP-HPLC analytical method, as well as its implementation in a manufacturing process.

2. Experimental

2.1. Materials, reagents and chemicals

The *Galanthus nivalis* lectin (GNL)- and Q-sepharose-purified HIV-1 CO6980v0c22 gp145 reference material (RM) expressed in CHO-K1 cells was obtained from Advanced Bioscience Laboratories (ABL Inc.). HIV-1 SF162 gp140 recombinant protein produced in HEK 293T cells [23–26] was provided by the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH. Acetonitrile, isopropanol, methanol, *n*-propanol, trifluoroacetic acid (TFA), and LC-MS grade water, were purchased from Honeywell (Charlotte, NC, USA). The Dulbecco's Phosphate-Buffered Saline (D-PBS, Corning, Corning, NY, USA) 1X without calcium and magnesium was obtained from VWR. CHO-K1 PC-2 spent medium was obtained from CDI Laboratories.

2.2. Preparation of standards and mobile phase

The CO6980v0c22 gp145 reference material (RM) in D-PBS 1X was used to prepare the standards for method development. Different concentrations of CO6980v0c22 gp145 RM (12.5, 25, 37.5, 50, 75, and 100 µg/mL) were diluted in 50% CHO-K1 spent medium (final concentration 50% D-PBS 1X and 50% CHO-K1 spent medium). The buffer blank was prepared by mixing D-PBS 1X and CHO-K1 spent medium 1:1. Mobile phase A consisted of 0.1% TFA and 2% *n*-propanol in LC-MS water and mobile phase B consisted of 0.1% TFA, 70% isopropanol, and 20% acetonitrile in LC-MS water.

2.3. Chromatographic conditions

Chromatographic separation was performed on an Agilent Technologies (Santa Clara, CA, USA) 1260 Infinity II BioInert HPLC quaternary pump system equipped with a diode array detector (DAD VL+). A reverse-phase C8 column (Zorbax 300SB-C8 Rapid Resolution 2.1 × 50 mm, 3.5 µm) and C8 guard column (Zorbax 300SB-C8, 2.1 × 12.5 mm, 5 µm) were used. The following gradient elution was used (time/%B): 0/30, 2/30, 3.5/65, 4/70, 5/75, 6.5/95, 9.5/95, and 10/30, with a post time of 5 min. The following HPLC running conditions were used: injection volume was 40 µL, flow rate was maintained at 0.4 mL/min, detection was performed at 280 nm (with a reference wavelength set at 320 nm) and column temperature was set to 80 °C. To reduce the carry over, 100 µL of D-PBS 1X was injected between sample or standard injections.

2.4. Evaluation of the RP-HPLC method against ICH guidelines

The RP-HPLC method for the quantitation of CO6980v0c22 gp145 was evaluated against the current criteria for linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, repeatability, and robustness of the ICH guidelines Q2 (R1) (ICH Guidelines, 2005). The LOD and LOQ were calculated using the following equations, respectively, $LOD = 3.3{*}\sigma/S$ and $LOQ = 10{*}\ \sigma/S$, where σ is the standard deviation of intercept and S is the slope of the linear regression curve.

2.5. Cell culture and western blotting

Stably transfected CHO-K1 cells expressing CO6980v0c22 gp145 were grown in PowerCHO 2 (Lonza, Basel, Switzerland) medium supplemented with 10 µg/ml puromycin, 4 mM glutaMAX™ and 1% penicillin/streptomycin. A seeding density of 0.8 million cells/mL was used to inoculate a 40 L vessel controlled by a Finesse G3Lite (ThermoFisher Scientific, Waltham, MA, USA) system with an agitation of 65 rpm, dissolved oxygen 35%, pH 6.8 with CO_2 adjustment, and a reactor temperature of 37 °C. Daily culture samples was collected from the bioreactor for CO6980v0c22 gp145 daily titer analysis. The samples were mixed 1:1 with gel sample buffer and analyzed by SDS-PAGE using 4–20% acrylamide (Invitrogen, ThermoFisher, Waltham, MA, USA). A total of 1.0, 0.5, 0.25, and 0.125 µg was loaded of the reference standards for quantification. For western blot analysis, the gel was

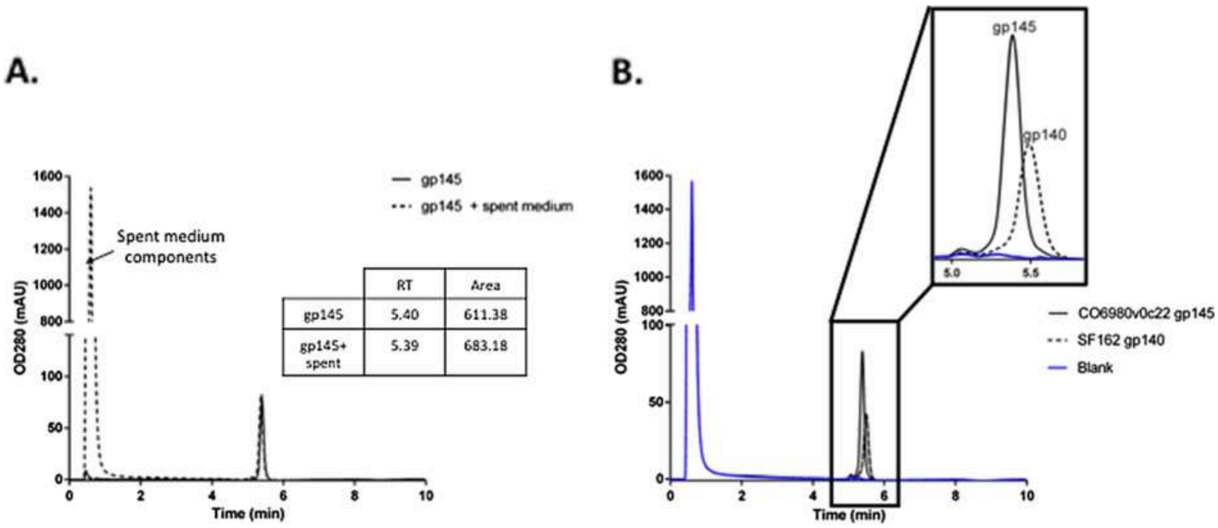

Fig. 2. RP-HPLC method specificity. (A) Representative chromatograms obtained from purified gp145 (60 μg/mL in D-PBS) alone and gp145 spiked into spent medium (60 μg/mL in 50% D-PBS and 50% PC2 spent medium). (B) Representative chromatograms obtained from three (3) injections of the HIV-1 CO6980v0c22 gp145 RM in 50% 1X D-PBS and 50% spent medium (60 μg/mL, black solid line), SF162 gp140 in 50% 1X D-PBS and 50% spent medium (60 μg/mL, black dashed line) and a buffer blank (blue solid line) onto a Zorbax 300SB-C8 rapid resolution RP-HPLC column (2.1 × 50 mm, 3.5 μm) using the Agilent BioInert Infinity II 1260 System. The zoom window highlights the peaks observed for the two HIV-1 Env proteins. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

transferred to a nitrocellulose membrane that was subsequently incubated with the broadly neutralizing antibody 4E10 (Polymun Scientific Immunbiologische Forschung GmbH, Klosterneuburg, Austria). The secondary labeling step was performed using an anti-human IgG coupled with alkaline phosphatase (ThermoFisher, Waltham, MA, USA). The resulting bands were visualized on a Chemidoc XRS+ (BioRad, Hercules, CA, USA) and quantified by densitometry using Image Lab™ Software from BioRad. Briefly, a linear standard curve was generated using the gp145 standards of known concentrations (1.0, 0.5, 0.25, and 0.125 μg).

3. Results

3.1. Reversed-phase HPLC method development

Several parameters were tested and optimized during the development of the RP-HPLC method for the determination of gp145 titer from bioreactor supernatants. The most noteworthy changes that resulted in substantial improvements were in sample preparation and gradient elution schemes. Two specific conditions are described here to illustrate how the method was improved (Fig. 1). Condition A involved the dilution of sample in 40% D-PBS, 40% Mobile phase A, and 20% CHO-K1 spent medium with a “fast” gradient (30%B => 65%B in 30 s), whereas Condition B involved the dilution of sample in 50% D-PBS and 50% CHO-K1 spent medium with a slow gradient (30%B => 65%B in 90 s) The results show that the peak shape and recovery were improved as indicated by tail factor (TF) and area (AUP), respectively. The tail factors observed were 2.333 and 1.057 for Condition A and Condition B, respectively. Furthermore, the area under the curve (AUP) for each peak was 738 and 1115 for Conditions A and B respectively. Altogether these results indicated that Condition B was adequate for the titer determination of gp145 supernatants.

3.2. Method specificity

To evaluate the specificity of our analytical method, the spent medium alone (buffer blank) and the spent medium spiked with CO6980v0c22 HIV-1 gp145 were analyzed by RP-HPLC. The standards used were mixed 1:1 with PC2 spent medium to best mimic the sample matrix that we intend to quantify, namely, gp145 supernatants directly from bioreactors. Three consecutive injections of 40 μL were analyzed by RP-HPLC. Peaks arising from spent medium components are only observed between 0 and 2 min (Fig. 2). There is no signal observed from the sample matrix in the region where gp145 elutes, from minute 5 to 6. Thus, the spent medium does not interfere with the measurements of gp145. Since the data show that the gp145 peak is clearly resolved from the spent medium peak, this indicates that the method is specific for gp145 (Fig. 2).

To further challenge our analytical method, gp145 (clade C) and SF162 gp140 (clade B) were analyzed by RP-HPLC. Retention times of 5.385 min and 5.489 min were observed for CO6980v0c22 gp145 and SF162 gp140, respectively (Fig. 2B, Table 1). This data show that our RP-HPLC method can discriminate between these two very similar HIV-1 Env immunogens: CO6980v0c22 gp145 and SF162 gp140.

Table 1
Retention parameters of the HIV-1 env proteins.

Sample	Retention time (average, minutes)	STDEV	% RSD
CO6980v0c22 gp145	5.385	0.001	0.2
SF162 gp140	5.489	0.001	0.2

Table 2
Validation parameters for the RP-HPLC analysis of CO6980v0c22 gp145.

Parameter	Value
Concentration Range	12.5–100 μg/mL
Intercept	17.79
Coefficient of Determination (R^2)	0.9996
Standard Error of Intercept	3.184
Standard Deviation of Intercept	7.8
Limit of Detection (LOD)	2.4 μg/mL
Limit of Quantitation (LOQ)	7.1 μg/mL

3.3. Linearity and range

A series of CO6980v0c22 HIV-1 gp145 standard samples, ranging in concentration from 12.5 to 100 μg/mL, each in 50% D-PBS 1X and 50% PC2 spent medium, was injected sequentially into the HPLC system. The

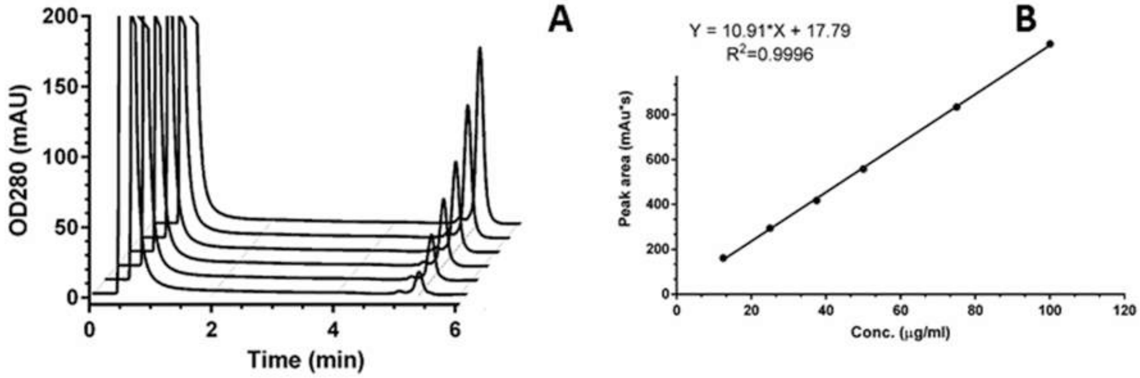

Fig. 3. Standard curve for CO6980v0c22 HIV-1 gp145 reference material. Samples of reference material in 50% 1X D-PBS and 50% CHO-K1 spent media were injected into the RP-HPLC column. (A) Chromatograms represent the single peak for each of the CO6980v0c22 gp145 RM standards at concentrations of 12.5, 25, 37.5, 50, 75, and 100 μg/mL. (B) The area under the curve for each standard injection was plotted as a function of the known concentration. Injections were performed in triplicate and the average of the three injections is reported.

Table 3
Accuracy of the RP-HPLC method for the quantitation of CO6980v0c22 gp145.

Standard theoretical concentration (μg/mL)	Standard 1 (AUP) 12.5	Standard 2 (AUP) 25	Standard 3 (AUP) 37.5	Standard 4 (AUP) 50	Standard 5 (AUP) 75	Standard 6 (AUP) 100
Replicate 1	159.688	298.354	416.166	557.712	832.336	1113.595
Replicate 2	160.957	299.258	417.580	557.202	833.186	1115.165
Replicate 3	162.059	285.307	419.959	558.925	835.098	1114.760
Mean of AUP	160.900	294.310	417.900	557.950	833.540	1114.510
% RSD	0.74	2.65	0.46	0.16	0.17	0.07
Calculated concentration (μg/μL)	13.1	25.3	36.7	49.5	74.8	100.5
% Recovery	104.9	101.4	97.8	99.0	99.7	100.5
% Recovery average: 100.56, STDEV: 2.44, %RSD: 2.42						

The area under the peak (AUP) was measured for 6 standards, each prepared from a known concentration of the reference material. Each point was determined in triplicates. Recovery is expressed as: (calculated conc./theoretical conc.)*100.

Table 4
Precision of the RP-HPLC method for the quantitation of CO6980v0c22 gp145.

	User #1	User #2
Standard theoretical concentration (μg/ml)	Standard 1 (AUP) 60	Standard 2 (AUP) 60
Replicate 1	669.39	683.508
Replicate 2	667.60	684.305
Replicate 3	665.96	681.727
Replicate 4	670.87	684.493
Replicate 5	671.88	684.057
Replicate 6	667.13	682.474
Average	668.80	683.43
Calculated Concentration (μg/ml)	59.660	60.872
% Recovery	99.43	101.45
% RSD	0.34	0.16
Overall % RSD	1.16	

Two different users performed the injection of a 60 μg/mL test sample six times. User #1 performed all injections on the same day, and User #2 performed the injections on a different day. The overall % RSD is the relative standard deviation of all injections performed.

area under the peak (AUP) was obtained for each sample and the mean and relative standard deviation (RSD) were calculated for each run. In addition, the slope, Y-intercept, coefficient of determination (R^2), LOD and LOQ were determined from the linear regression analysis. Results in Table 2 and Fig. 3 show the linear correlation between AUP and the concentration of CO6980v0c22 gp145 in the sample. The R^2 for the calibration curve shown in Fig. 2 was 0.9996 and the average for three independent experiments carried out on different days, is 0.997 with a standard deviation of 0.0002. Additional parameters of the regression equation are shown in Table 2.

3.4. Accuracy

The CO6980v0c22 gp145 concentrations of 12.5, 25, 37.5, 50, 75 and 100 μg/mL were used to determine the accuracy of the RP-HPLC method. The percent recovery was 100.56 ± 2.44 with a percent RSD below 2.7% (Table 3). Collectively, these results indicate that the RP-HPLC method described herein is suitable for the quantitation of CO6980v0c22 gp145 directly from bioreactor supernatants.

3.5. Repeatability

The repeatability (inter-day variation) was determined from the results from six independent injections of a sample containing 60 μg/mL of CO6980v0c22 gp145 RM (Table 4). The concentration of 60 μg/mL was chosen because it lies in the middle of the linear range. Results show a % RSD of 0.34 and 0.16 for the inter-day variations for USER #1 and #2, respectively. Moreover, for the intermediate precision, the differences between different users were recorded and evaluated (Table 4). The experiments were carried out on different days and with fresh solvent each day. The overall %RSD for the intermediate precision is 1.16.

3.6. Robustness

Method robustness was assessed by testing the following parameters: column temperature, column ageing, guard column lot variations, and stability of the gp145 material. For the column ageing, the RP-HPLC method was carried out after 6 months of use and after 1742 injections. Fresh solvent was used each time. Results show an overall %

Table 5
Robustness (column ageing) of the RP-HPLC method for CO6980v0c22 gp145.

Concentration of gp145 standard	AUP Month 1	Mean of AUP	% RSD	AUP Month 6	Mean of AUP	% RSD	Overall %RSD
12.5 μg/mL	159.688 160.957 162.059	160.90	0.74	148.379 149.275 149.320	148.99	0.36	5.44
25 μg/mL	298.354 299.258 285.307	294.31	2.65	278.774 280.368 279.369	279.50	0.29	3.65
37.5 μg/mL	416.166 417.58 419.959	417.90	0.46	411.009 411.727 406.483	409.74	0.69	1.39
50 μg/mL	557.712 557.202 558.925	557.95	0.16	552.539 551.055 558.947	554.18	0.76	0.48
75 μg/mL	832.336 833.186 835.098	833.54	0.17	824.642 820.339 815.179	820.05	0.58	1.15
100 μg/mL	1113.595 1115.165 1114.76	1114.51	0.07	1089.800 1086.568 1093.274	1089.88	0.31	1.64
Coefficient of Determination (R^2): Month 1: 0.9996, Month 2: 0.9998							

All standard concentrations were analyzed in triplicates on different months. The column and the HPLC system remained in use between Month 1 and Month 6. The overall % RSD is the relative standard deviation of all injections carried out for that standard concentration.

Table 6
Effect of the guard column on the analysis of CO6980v0c22 gp145.

Sample Concentration	AUP for Guard Column #1 Lot# USGH002312	Avg.	% RSD	AUP for Guard Column #2 Lot# USGH002317	Avg.	% RSD	Overall % RSD
60 μg/mL	646.399 651.615 652.303	650.11	0.5	657.629 663.513 660.904	660.48	0.45	1.14

Two different guard columns were used in the injection of a standard of known concentration. The overall % RSD is the relative standard deviation of all injections under both guard columns.

Table 7
Effect of column temperature on the precision.

	Temperature °C			Overall % RSD
	78	80	82	
AUP	683.690 684.480 684.228	689.676 692.576 692.087	675.040 675.157 672.157	1.11
Retention Time	5.354 5.353 5.354	5.331 5.331 5.331	5.309 5.311 5.309	0.36

The overall % RSD is the relative standard deviation of all injections at all temperatures.

Table 8
Ageing effect of CO6980v0c22 gp145 at 10 °C.

Day	Average retention time ± STDEV (min)	Mean AUP	STDEV	Overall Mean AUP % RSD
0	5.331 ± 0.002	680.44	1.46	2.73
1	5.321 ± 0.003	650.11	3.23	
2	5.320 ± 0.002	663.57	6.29	
3	5.311 ± 0.002	638.68	30.83	

A test sample (60 μg/mL) was incubated at 10°C for 3 days and analyzed in triplicates. The Overall Mean AUP % RSD is the relative standard deviation of the peak intensities for all injections performed as part of this stability study. The same mobile phase was used throughout this analysis.

RSD below 6 for the CO6980v0c22 gp145 concentrations of 12.5, 25, 37.5, 50, 75, 100 μg/mL (Table 5). The overall percent RSD observed for variations in the guard column lots and column temperature (78 °C, 80 °C, and 82 °C) were 1.14% and 1.11%, respectively (Tables 6 and 7). Moreover, age effects on mobile phase and test sample analysis shows that mobile phase and CO6980v0c22 gp145 solutions are stable for 4 days at 10 °C with an overall % RSD of 2.73% (Table 8).

3.7. Quantitation of HIV-1 CO6980v0c22 gp145 from bioreactors

To challenge the newly developed RP-HPLC method, actual supernatants from a bioreactor run were analyzed and compared against quantification by Western blot analysis. Samples of supernatant from CHO-K1 cells expressing CO6980v0c22 gp145 were removed daily from a 40 L bioreactor and analyzed by RP-HPLC and western blot (Fig. 4). The concentration estimates obtained by western blot were consistently higher than the RP-HPLC measurements. The concentrations measurements by RP-HPLC were performed in triplicates and the average standard deviation was determined to be less than 4% of the total value. A representative chromatogram of the gp145 protein obtained from cell culture supernatant is shown in Fig. 5.

4. Discussion

The production of protein-based vaccines in bioreactors requires the development of methods for the quantitation of the desired product, that are quick, highly selective, accurate, reproducible, and that require minimal sample processing. The production of HIV Env vaccines mainly employs ELISA for the quantification of products secreted into the cultivation media. While ELISA is inexpensive and relatively easy to

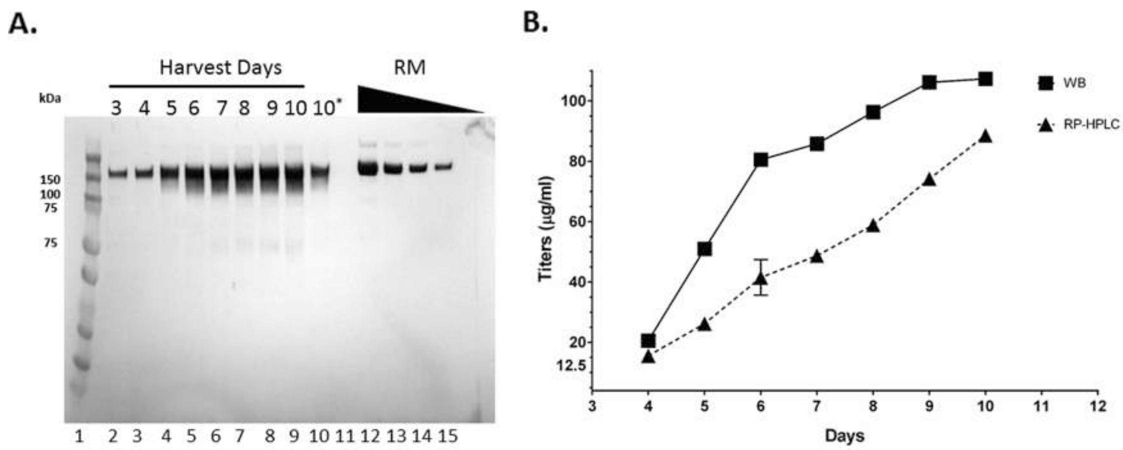

Fig. 4. Quantification of CO6980v0c22 gp145 from a bioreactor. A CHO-K1 cell line expressing CO6980v0c22 gp145 was grown in a 40 L bioreactor from which samples were removed daily and diluted 1:1 with 1X D-PBS, prior to RP-HPLC analysis. (A) An initial verification of expression was carried out by Western blot (WB). The intensity of the WB bands for the culture supernatants (lanes 2–10, each lane contains 12 μL of supernatant) was compared to that of standards of known concentrations, 1.0, 0.5, 0.25, and 0.125 μg (lanes 12–15). The asterisk (*) indicates the day 10 sample diluted 1:3. (B) The same samples analyzed by RP-HPLC. The concentrations were plotted to show the daily increase in CO6980v0c22 gp145 production until harvest.

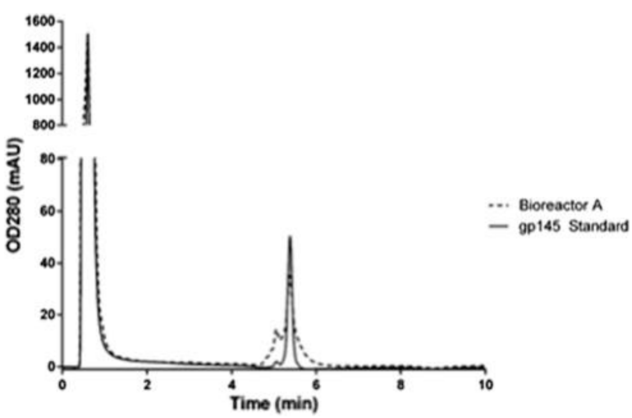

Fig. 5. Representative chromatogram of CO6980v0c22 gp145 from a bioreactor supernatant. A CHO-K1 cell line expressing CO6980v0c22 gp145 was grown in a bioreactor from which a supernatant sample was collected and diluted 1:1 with 1X D-PBS, prior to RP-HPLC analysis. The gp145 RM standard has a final concentration of 37.5 μg/mL and is in 50% D-PBS and 50% PC2 spent medium.

implement in any production setting, the waiting times for ELISA incubations and washes can be long and the variability of the measurement can often be too wide for the method to be considered accurate, depending on the antibodies and standards used [27]. Another method for the detection and quantitation of protein products in crude supernatants is the Western blot analysis, which also depends on the recognition of the protein analyte by a primary antibody. The high variability associated with both ELISA and Western blot may arise from their reliance on the quality and specificity of the antibodies used for detection [28].

Here, we report a RP-HPLC method for the quantification of an HIV-1 CO6980v0c22 gp145 vaccine candidate directly from culture supernatants of CHO-K1 cells that does not rely on antibody binding. This method is specific since the addition of CHO PC2 spent medium does not interfere with the quantification analysis of gp145. Moreover, we observed a peak corresponding to the medium components in the first 2 min of the gradient elution, which may arise from aromatic amino acids, such as tyrosine and tryptophan, but this did not interfere or alter the signal observed for gp145 in the 5–6 min time frame. The method described herein was found to be linear within a broad range of concentrations, with a standard deviation within 3%, and an average recovery of 101%. The method was accurate, with 2.7% variability on consecutive measurements. The method was precise, with an inter-day variability of 0.34% and an inter-user variability of 1.2% for two different users. The method was also found to be robust, as the measurements obtained with a 6-month old column (after 1742 injections) were within 5.4%–1.6% of the measurements obtained with a new column. The use of different guard columns did not greatly affect separation, since the guard column variability was found to be 1.1%. Finally, the same measurement was carried out with a standard that had been left at 10 °C for 3 days and the measurements differed by less than 2.7%, providing a first insight into the effects of ageing on this highly glycosylated family of vaccines.

A direct comparison between the RP-HPLC method and Western blot analysis indicates that our method is suitable for the determination, with low variability, of protein concentration in a bioreactor (Fig. 4). Our RP-HPLC method registered the daily increase in the concentration of CO6980v0c22 gp145 in the bioreactor, with a variability of approximately 4% of the total value. The Western blot method, however, consistently gave higher concentration values, a commonly observed phenomenon for which we have no explanation, although the limitations of the Western blot as a quantitative technique have been extensively reported [28].

5. Conclusion

In all, we have presented the development of a RP-HPLC method for the detection and quantification of HIV-1 CO6980v0c22 gp145 in CHO-K1 culture supernatants. This method could be easily adapted for the analysis of other glycoproteins produced in mammalian cell systems. Our method was determined to be accurate, precise, robust, and required a running time of 20 min per sample, substantially shorter than the time needed for an ELISA measurement.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2021.122562.

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