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RESEARCH

A continued process verification strategy at first stages of monoclonal antibody purification by integrated risk assessment and multivariate data analysis

✍Arturo Toledo Rivero[1], Osvaldo Gozá León[2], Erick Hernández León[1], Idrissa Leonard Rupalé[3], Giselle Hidalgo Márquez[2]

[1] Quality Department at Research and Development, Center of Molecular Immunology, CIM 216 st and 15 Ave, Atabey, POBox 11600, Havana, Cuba
[2] Faculty of Chemical Engineering, Technological University of Havana José Antonio Echeverría, CUJAE 114 st, No. 11901, Marianao, POBox 19390, Havana, Cuba
[3] Quality Department at ANTYTER Facility, Center of Molecular Immunology, CIM
✍arturot@cim.sld.cu

ABSTRACT

According to current regulatory expectations, continued process verification (CPV) must guarantee post-qualification monitoring of critical process parameters (CPP). Such parameters are not easy identifiable in biotechnological processes given its inherent complexities. Therefore, this work was aimed to bring methods for an effective determination of CPP thus providing the necessary groundwork for elaborating a CPV strategy. Knowledge and experience accumulated along the lifecycle of a legacy monoclonal antibody product were applied, focusing on its first stages of downstream purification. Process parameters defined for analysis were ranked through a cause-effect risk matrix and criticality levels deduced using Pareto distribution. Subsequently, data from three consecutive production campaigns were processed by the principal component analysis (PCA) method for a comprehensive characterization of process variability, as well as clusters analysis and soft independent modeling of class analogy (SIMCA) methods for differentiating operational modes among campaigns. A set of 13 process parameters were confirmed as CPP, given its major impact on process variability, while the remaining five were considered as key operating parameters (KOP). Such outcome, achieved theoretically, was corroborated with process actual operational incidences, contributing to elaborate a well-founded monitoring plan for assuring CPV and its viable execution.

Keywords: monoclonal antibody purification, continued process verification, cause-effect risk matrix, principal component analysis, cluster analysis, soft independent modelling of class analogy

RESUMEN

Estrategia de verificación continuada del proceso en las primeras etapas de la purificación de un anticuerpo monoclonal mediante la integración del análisis de riesgo y el análisis multivariado de datos. De acuerdo a las expectativas regulatorias actuales, la verificación continuada del proceso (VCP) debe asegurar el monitoreo post-calificación de los parámetros críticos del proceso (PCP). Estos no son fácilmente identificables en los procesos biotecnológicos dadas sus inherentes complejidades. Ante esta problemática, el presente trabajo está dirigido a aportar métodos para la efectiva determinación de estos PCP, proporcionando así las bases necesarias para la elaboración de una sólida estrategia de VCP. Se integró el conocimiento y la experiencia acumulados durante el ciclo de vida de un producto monoclonal ya registrado bajo estándares anteriores. La estrategia se enfocó a las primeras etapas de purificación, cuyos parámetros del proceso definidos para el análisis fueron categorizados mediante una matriz de riesgo de causa-efecto y los niveles de criticidad deducidos utilizando el criterio de Pareto. Seguidamente, se procesaron los datos de tres campañas productivas mediante el método de análisis de componentes principales (ACP) para una caracterización integral de la variabilidad del proceso. También se aplicó los métodos de análisis de clústeres, y de modelación independiente y flexible de analogía de clases (MIFAC) para la diferenciación de modos operacionales entre campañas. Se logró confirmar un grupo de 13 parámetros críticos por su mayor impacto en la variabilidad del proceso, mientras que los cinco parámetros restantes fueron considerados como parámetros operacionales claves (POC). Dicho resultado, logrado sobre bases teóricas, fue corroborado con las incidencias operativas reales del proceso, y contribuyó a la elaboración de un plan de monitoreo bien fundamentado para asegurar la VCP y su ejecución viable.

Palabras clave: purificación de anticuerpos monoclonales, verificación continuada del proceso, matriz de riesgos de causa-efecto, análisis de componentes principales, análisis de clústeres, modelación independiente y flexible de analogía de clases

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Introduction

Process validation have been defined by the main regulatory agencies (i.e., Food and Drug Administration (FDA) and the European Medicines Agency (EMA)) as a three stages exercise in full correspondence with product and manufacturing process lifecycle [1-4]. They have also encouraged the application of quality risk management and most modern development principles.

1. FDA Guidance for industry. Process validation: general principles and practice. U.S. Department of Health and Human Services. January 2011.

In such renovated concept, continued process verification (CPV) last stage has received special attention to continuously guarantee that the process remains under control during routine production. Therefore, there is a need of implementing an effective post-qualification monitoring of process parameters, with significant impact on product's critical quality attributes (CQA) [5]. However, these parameters are not so easy to identify in highly complex processes as those applied in the biotechnological field [6]. In fact, there are still manufacturers that fail to implement CPV as can be seen in the significant amount of FDA's warning letters, due to poor critical process parameters (CPP) identification derived from insufficient process understanding [7].

There are examples on using risk models and design of experiments (DoE) for such identification mainly at the first stage of process development [8-10], or even at later stages taking advantage of the long history from developing to manufacturing, as it is the case of legacy products [11, 12]. Moreover, the multivariate nature characterizing most biotechnological unit operations is an unquestionable issue to deal with, as substantial correlations among process parameters and other difficulties related to handling large volume of data limit the traditional statistic's application. These explain the increasing relevance that multivariate data analysis (MVDA) approach has acquired in the biotechnology and biopharmaceutical domain [13, 14]. Several applications have been described in this field, such as omics at the development stage, where MVDA provides the tools for a significant complexity reduction in data processing [15-18]. Yet, there are a discrete number of published references dealing with process characterization at the commercial manufacturing stage [19, 20].

Therefore, the present work was aimed to provide practical means to overcome such difficulties inherent to CPP determination in an existing downstream first purification process of a legacy monoclonal antibody commercial product (mAb). It showed the advantages of integrating process knowledge and experience throughout product lifecycle in a risk influence matrix model, along with data processing from annual production campaigns based on MVDA tools.

Materials and methods

Description of purification stages under study

Following a prescribed strategy to cope with a potential increase of mAb production, the present work focused on downstream purification first stages within bulk manufacturing process, technologically adapted to guarantee a successful performance on final product yield and the removal of most impurities [21]. The implemented configuration is shown in figure 1, where first purification unit operations consist of IgG capture by protein A chromatographic affinity (Novasep, France) followed by elution and anion exchange using chromatographic membranes (Sartorius, Germany) as main steps, which also include acid viral inactivation and pH/conductivity adjustment intermediate operations before anion exchange. Due to batch mode predominance, a certain IgG mass input is required by harvest volume pooling, in order to guarantee the efficient operation of protein A column matrices.

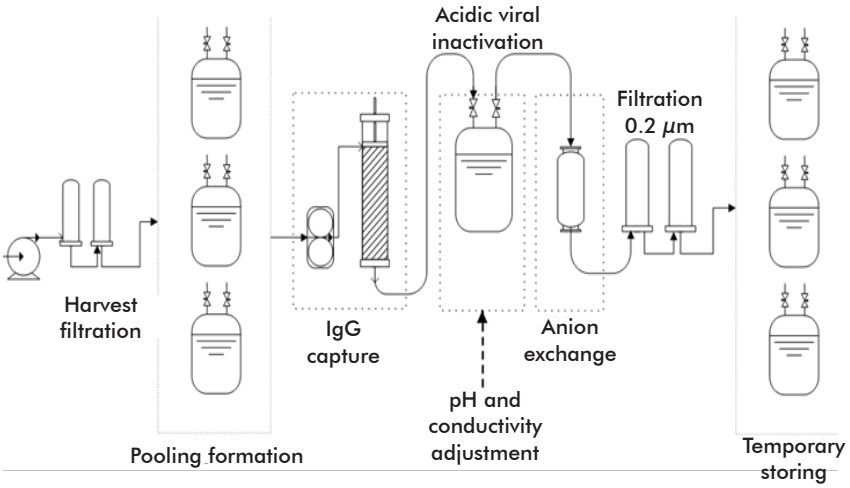

Figure 1. Downstream purification first stages flow diagram.

Cause-effect risk matrix and Pareto methods

For performing this exercise, a working group of three specialists of high and adequate professional degree (PhD.) and more than 10 years of experience in downstream processing was established. They contributed with their knowledge assimilated throughout product/process lifecycle, to criticality assess of bulk final product quality attributes, as well as impact of process parameters on them. Guided by Mitchell's work [9], index allocation tables were previously elaborated under consensus of the working group in order to facilitate evaluation, as shown in table 1.

2. EMA Guidance. Process validation for the manufacture of biotechnology-derived active substances and data to be provided in regulatory submissions. Committee for Medicinal Products for Human Use. April 2016.

3. EU Guidelines for GMP. Qualification and validation. EUDRALEX Volume 4. Annex 15. March 2015.

4. Castillo FC, Cooney B, Levine HL. Biopharmaceutical manufacturing process validation and quality risk management. Pharm Eng. 2016;36(3):82-92.

5. DiMartino M, Zamamiri A, Pipkins K, Heimbach J, Hamann E, Adhibhatta S, *et al*. CPV signal responses in the biopharmaceutical industry. Pharm Eng. 2017;37(1):57-64.

Table 1. Index allocation tables for quality attributes and process parameters risk assessment

Assessment criteria regarding the effect of quality attributes variability on patient safety and effectiveness	Criticality	Index
Failure to comply specifications causes adverse reactions of very high consideration, or there is not therapeutic action at all for which the product is prescribed. Patient safety is compromised.	Very high	7
Failure to comply specifications causes adverse reactions of some significance, but tolerable for the patient, or there is some therapeutic action for which the product was prescribed but still insufficient. Patient safety is marginally compromised.	High	5
Failure to comply specifications leads to secondary effects causing discomfort that can be commonly assimilated, or there is limited therapeutic action. Patient safety is not compromised at all.	Moderate	3
Failure to comply specifications produces minor effects of very low consideration without complications. Patient safety is definitely not compromised.	Low	1

Assessment criteria regarding the effect of process parameters and/or variables on quality attributes	Impact	Index
Variability affects directly on quality attributes, even bypassing intermediate process steps where there are no transformations related to the parameter and/or variable in question. There are evidences and/or experience that a high interrelation exists.	High	7
Variability impacts indirectly on quality attributes, as it can affect process intermediate steps that subsequently can have substantial influence on these. There are evidences and/or experience that a significant interrelation still exists.	Middle	5
Variability affects process intermediate steps, but with little or no incidence on quality attributes. There are evidences and/or experience that interrelation is poor.	Low	3
Variability has low impact on intermediate process steps without affecting any quality attribute. There are evidences and/or experience that there is not any interrelation.	None	1

Information obtained from the numeric assessment was then incorporated into the cause-effect risk matrix as represented in table 2. Thus, the impact of each operation parameter in the corresponding steps/unit operations could be numerically evaluated as a contribution to each CQA variability for determining a total rank index and next, rank indexes percentage from the grand total.

A Pareto chart was subsequently elaborated by plotting rank indexes for each parameter in descending order at one side, and at the other side the cumulative index rank percentage. Then, the well-known Pareto criterion 80-20 % was applied for obtaining a set of parameters with higher criticality, regarding impact on process variability for each purification step, which can be taken at first as a deduced design space.

Multivariate methods for data analysis

Data collected from three consecutive annual manufacturing campaigns (identified Y1, Y2 and Y3) since startup of the referred technological configuration were arranged in a single working matrix of 1924 elements, where rows represent the first purification intermediate batches and columns represent process parameters formerly assessed as critical.

Given the considerable difference in range and magnitude among parameters, range normalization was applied by rows, as well as auto-scaling standardization by columns (ratio of centered mean and the standard deviation) in order to avoid any prevalence [22].

On this basis, principal component analysis (PCA) was applied to work matrix providing dimensionality reduction in a few independent latent variables or principal components (PC), thus facilitating verification of true impact of referred parameters on process variability, as well as possible correlation between each other and identification of score outliers based on Hotelling's T^2 criterion representing batch unusual operations [22]. Likewise, PCA scores chart facilitated identification of batch variability patterns of annual production campaigns in combination with cluster analysis [23] as unsupervised classification procedure, allowing distinguishing between operational modes to some extent. Furthermore, to reaffirm this differentiation, a supervised classification method such as soft independent modeling of class analogies (SIMCA) was applied [24].

The UNSCRAMBLER X version 10.4 software was used to run the above multivariate data analysis methods, which does not mean a preference among other computer applications.

Results and discussion

Process parameters criticality assessment

After a thorough process analysis carried out by the working group, a set of 18 process parameters came out with relative importance in first purification unit operations and hence considered as significant in process performance. Such exercise included former experience in full-scale commercial production and scale-down studies based on IgG capture and anion exchange modifications for technological improvement. These parameters are summarized in table 3, with their corresponding description and abbreviations.

Table 2. Cause-effect risk matrix tabular model

Quality attributes	QA1	QA2	...	QAn	Total	%Rank
Criticality level	N1	N2	...	Nn		
Process Step/Unit Operation 1						
Parameter 1	M1	M2	...	Mn	T1	PcR1
Parameter 2	:	:	:	:	:	:
:	:	:	:	:	:	:
Process Step/Unit Operation 2						
Parameter 3	:	:	:	:	:	:
Parameter 4	:	:	:	:	:	:
:	:	:	:	:	:	:
Process Step/Unit Operation P						
:	:	:	:	:	:	:
Grand total					TT	100

* T1 was calculated by the formula: T1 = N1×M1 + N2×M2 +…. Nn×Mn. PcR1 was calculated as: PcR1 = T1/TT ×100.

Table 3. Prior identification of first purification process parameters

No.	Description	Id.	Purification step
1	Flow of adjusted protein A eluate to anion exchange	FlSQ	Anion exchange in chromatographic membrane
2	Conductivity of adjusted protein A eluate for anion exchange inlet	CoSQ	Anion exchange in chromatographic membrane
3	Conductivity of buffer solution for anion exchange membrane equilibrium	CoEQ	Anion exchange in chromatographic membrane
4	Flow of buffer solution for anion exchange membrane equilibrium	FlEQ	Anion exchange in chromatographic membrane
5	Conductivity of solution for eluate adjustment	CoAD	Eluate adjustment
6	Time of viral inactivation	TINV	Acidic viral inactivation
7	Mass of IgG in protein A eluate	MPAE	Elution of protein A column
8	pH of eluate from protein A column	pHEL	Elution of protein A column
9	pH of elution solution	pHES	Elution of protein A column
10	Flow of elution solution	FlES	Elution of protein A column
11	Third wash flow of buffer solution	FlW3	Third wash of protein A column
12	pH of second buffer solution	pHT5	Second wash of protein A column
13	Conductivity of second buffer solution	CoT5	Second wash of protein A column
14	Second wash flow of buffer solution	FlW2	Second wash of protein A column
15	First wash flow of buffer solution	FlW1	First wash of protein A column
16	Mass of IgG in filtrated harvest pool inlet	MIgG	Purification process input
17	pH of first buffer solution	pHT1	Equilibrium, first and third wash of protein A column
18	Flow of buffer solution for protein A column equilibrium	FlTE	Equilibrium of protein A column

Subsequently, cause-effect risk matrix parameters were ranked and Pareto chart criticality assessed as illustrated in figure 2. As shown, 13 parameters were regarded of greater impact in process variability, which represent a practical approximation of design space characterizing the first purification process. They were in descending order of relevance: pH of eluate from protein A column (pHEL), Conductivity of adjusted protein A eluate for anion exchange inlet (CoSQ), Flow of adjusted protein A eluate to anion exchange (FlSQ), pH of elution solution (pHES), Time of viral inactivation (TINV), Flow of elution solution (FlES), Mass of IgG in filtrated harvest pool inlet (MIgG), pH of second buffer solution (pHT5), Conductivity of second buffer solution (CoT5), Second wash flow of buffer solution (FlW2), First wash flow of buffer solution (FlW1), Conductivity of solution for eluate adjustment (CoAD) and pH of first buffer solution (pHT1).

6. Demmon S, Bhargava S, Ciolek D, Haley J, Jaya N, Joubert MK, *et al*. A cross-industry forum on benchmarking critical quality attribute identification and linkage to process characterization studies. Biologicals. 2020;67:9-20.

7. Pazhayattil A, Sayeed-Desta N, Ingram M. Lifecycle-based process validation emphasizes the need for continued process verification. Pharm. Tech. 2018 Supplement;(3):s22-5.

8. Rudge S. Quality risk management. Presentation at Center of Excellence Biopharmaceutical Technology Course Series; 2016 December 12 – 14. New Delhi: Indian Institute of Technology at New Delhi, India; 2016.

9. Mitchell M. Determining criticality-process parameters and quality attributes: criticality as a continuum. In: QbD and PAT in biopharmaceutical development. E-book presented in partnership with BioPharm Int. September 2017. p. 3-12.

10. Hakemeyer C, McKnight N, St. John R, Meier S, Trexler-Schmidt M, Kelley B, *et al*. Process characterization and design space definition. Biologicals. 2016;44:306-18.

It should be noted that these parameters are mainly related to protein A chromatographic column wash and elution operations, as well as to chromatographic membrane anion exchange, which make them critical steps. Consequently, the remaining five, not having a potential impact on CQAs, can be considered as key operating parameters (KOP) with influence just on process performance [9]. They consisted on: Conductivity of buffer solution for anion exchange membrane equilibrium (CoEQ), Flow of buffer solution for anion exchange membrane equilibrium (FlEQ), Third wash flow of buffer solution (FlW3), Flow of buffer solution for protein A column equilibrium (FlTE) and Mass of IgG in protein A eluate (MPAE). (Table 3).

Data structure modeling through PCA

Descriptive statistics and run charts applied preliminarily to data (charts not shown but available) revealed that process parameters behave differently concerning their relative variability. Flows passing through chromatographic column matrices are fixed by automatic means in order to achieve an operational residence time for efficient exchange. Nevertheless, they are set differently from batch to batch, most probably conditioned to MIgG entering the process, and these parameters are those that exhibited the greatest variability in the process. It is followed in significance by FlSQ and those parameters associated to manual operations and offline measurements such as pH and conductivity of prepared buffer solutions. Concurrently, there are others with less variability as they are more easily controlled such as TINV and CoSQ, the latter through inline measurement means.

First PCA model obtained (charts not shown but available) could explain 81.2 % of process variance with three PCs and cumulative predictive capacity of 73.4 %. Coincidently, TINV and CoSQ parameters did not contribute significantly to explained variance, confirming what discussed before, so these can be discarded for reducing unnecessary noise, the same with five true outliers found representing unusual behaving batches [19, 20]. A descriptive summary of outliers found are shown in table 4, before and after model improvement.

PCA model restructured on this basis can explain 81.3 % of process variance and keeping an adequate fit with just two PCs, with a cumulative predictive capacity of 74.2 % according to validation variance computed through cross validation method, as can be seen in the explained variance graph in figure 3A. Moreover, the influence graph in Figure 3B shows six batches with a relative high leverage from model's center by taking into account Hotelling's T2 limit for regular behavior, while a single batch is far from model's adjusted hyperplane due to its relative high F-residuals variance, which is also reflected in Table 4. From the aforementioned, this enhanced version of PCA model can be considered as representative of data structure to an acceptable extent, and taken as a base for further analysis.

Regarding process parameters in the base model, most of them are grouped along PC1 axis with correlation loading coefficients of more than 0.5, and less than – 0.5 in the case of FlW2, while FLES and FlW1 are in the negative direction of PC2. This is better visualized in the correlation loadings graph in figure 4 where all points representing process parameters are inside the zone between the circles, which demarcate the condition of having a relevant impact to process variability.

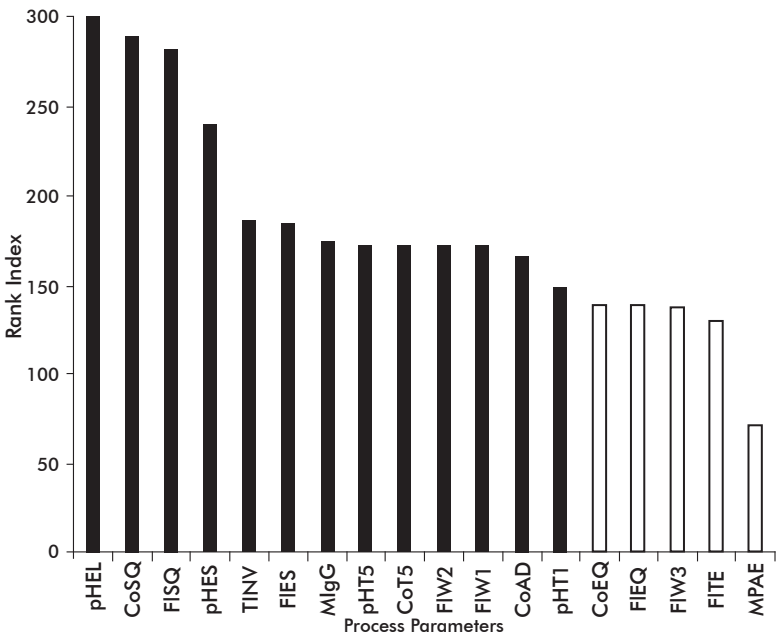

Figure 2. Pareto chart for first purification parameters criticality assessment. Bars in black stand for the most critical process parameters deduced from 80 % Pareto distribution according to cumulative percentage (81.2 %).

Table 4. Summary of outliers identified in annual campaigns

True outliers discarded to improve data structure model			
Campaign	Batch	Parameters involved	Associated operational event
Y2	13.06	MIgG	Low inlet IgG mass
		FlES	Low elution solution flow from capture
Y2	13.08	MIgG	Low inlet IgG mass
		FlES	Low elution solution flow from capture
Y2	13.33	pHT5	pH of second wash solution to chromatographic capture way over limit
		pHES	pH of eluate from capture slightly over limit
Y2	13.34	pHT5	pH of second wash solution to chromatographic capture way over limit
		pHES	pH of eluate from capture at the highest limit
Y3	14.06	FlW1	Low first wash solution flow to capture
		pHES	pH of eluate from capture slightly over limit
Outliers after improvement, only influential to data structure model			
Campaign	Batch	Parameters involved	Associated operational event
Y2	13.35	pHT5	pH of second wash solution to chromatographic capture way over limit
		pHES	pH of eluate from capture over limit
Y3	14.25	pHES	pH of eluate from capture over limit
Y3	14.33	pHES	pH of eluate from capture over limit
Y3	14.34	pHES	pH of eluate from capture over limit
Y3	14.54	pHES	pH of eluate from capture over limit
Y3	14.55	pHES	pH of eluate from capture over limit
Outliers after improvement, poorly described by data structure model			
Campaign	Batch	Parameters involved	Associated operational event
Y2	13.55	pHES	pH of eluate from capture over limit

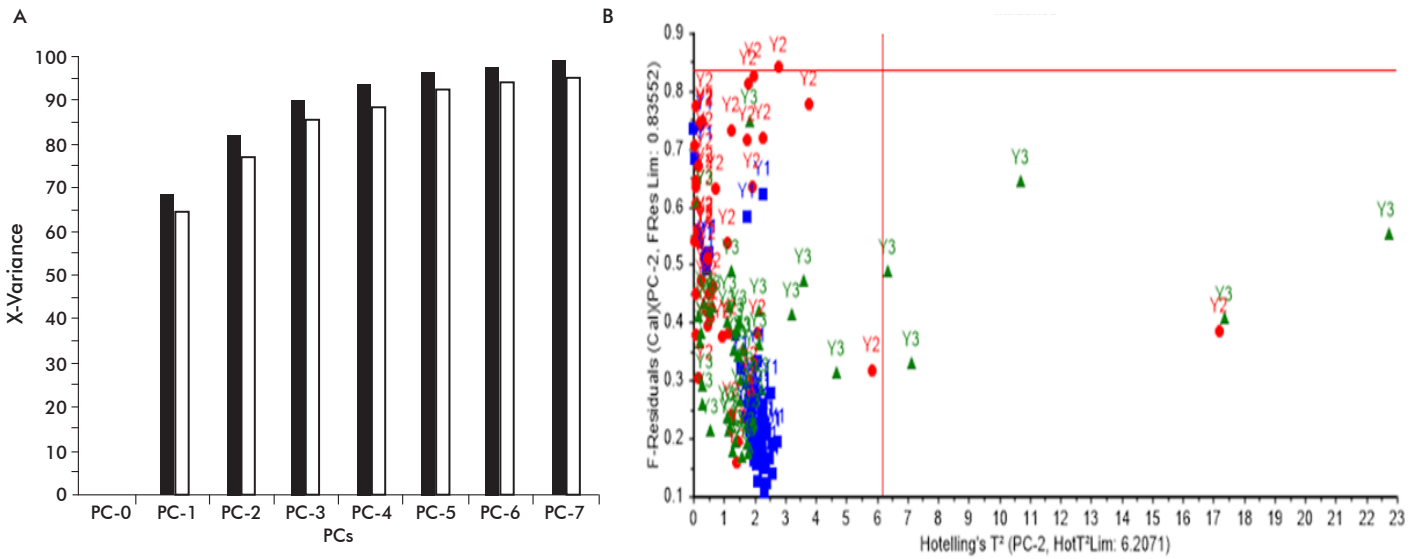

Figure 3. Graph's combination showing improved data structure PCA model fit. A) Explained variance of PCs. B) Influence graph of F-residuals vs. Hotelling's T^2

It should be taken into account that, since startup and in the course of productive campaigns there was a modest gradual increase of MIgG to first purification stage, yet with sustained fluctuations. At the same time, from Figure 4, it can be observed that it is inversely related to column wash and elution flows, where evidently operational considerations were draw in to guarantee a residence time increment as MIgG rises and vice versa.

It is also noted a group of parameters with similar contribution to PCs and visually close each other (highlighted with red ellipse), which denotes a significant correlation among them, for instance pHES and pHEL in the case of elution, pHES and pHT1 for first wash and so on. Furthermore, all solutions are formulated at very low ions concentration in pharmaceutical purified water, which explain pHT5 and CoT5 correlation in second wash, for instance. This should be examined under technological and operational considerations on a case-by-case basis in order to decide their role in CPV monitoring plan.

Likewise, process parameters are a reflection of operational modes changes along process campaigns from year to year. The best way to analyze this is to interpret side by side both correlation loadings charts and scatter plot scores representing batch trends, or by integrating both in a single bi-plot as shown in figure 5. At glance, first year campaign Y1 is mostly compromised to chromatographic washes and elution flow changes from batch to batch, which mark a difference with subsequent years Y2 – Y3. Second and third campaigns are more influenced with mIgG coming into the process, buffer solution parameters and FlSQ as well.

Batch operational modes' distinction through multivariate classification methods

Such differentiation was done by using the scores chart representing batch trends in figure 6, in combination with cluster analysis method (dendrogram not shown, summarized in table 5).

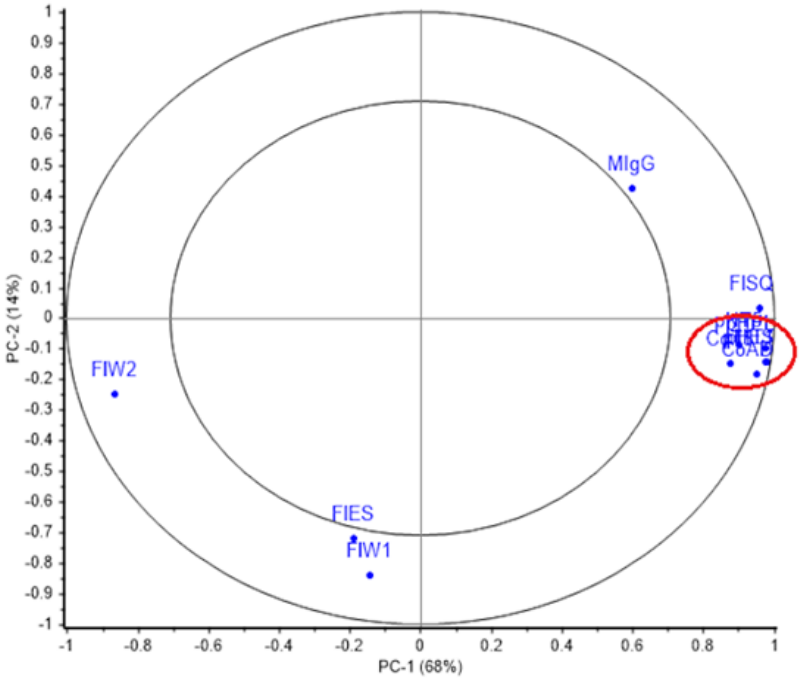

Figure 4. Correlation loadings graph showing parameter weighting coefficients on PCs.

Starting campaign in Y1 clearly shows a similar pattern where inter-batch adjustment of FlW2 for second wash predominates from 12.01 to 12.48, while end of the year batches 12.49 to 12.57 are far-off from that group. In these last, second wash operations set at lower rates prevail as a reaction to MIgG input instability and this without discarding the combined effect of other parameter changes. Then, the main cause of such batch operational mode trend lies in the staff's

11. Bika D, Butterell P, Walsh J, Epp K, Barrick J. Topic 2 – Stage 3 Process validation: applying continued process verification expectations to new and existing products. ISPE Discussion Paper 2012; August 1: p.1-36.

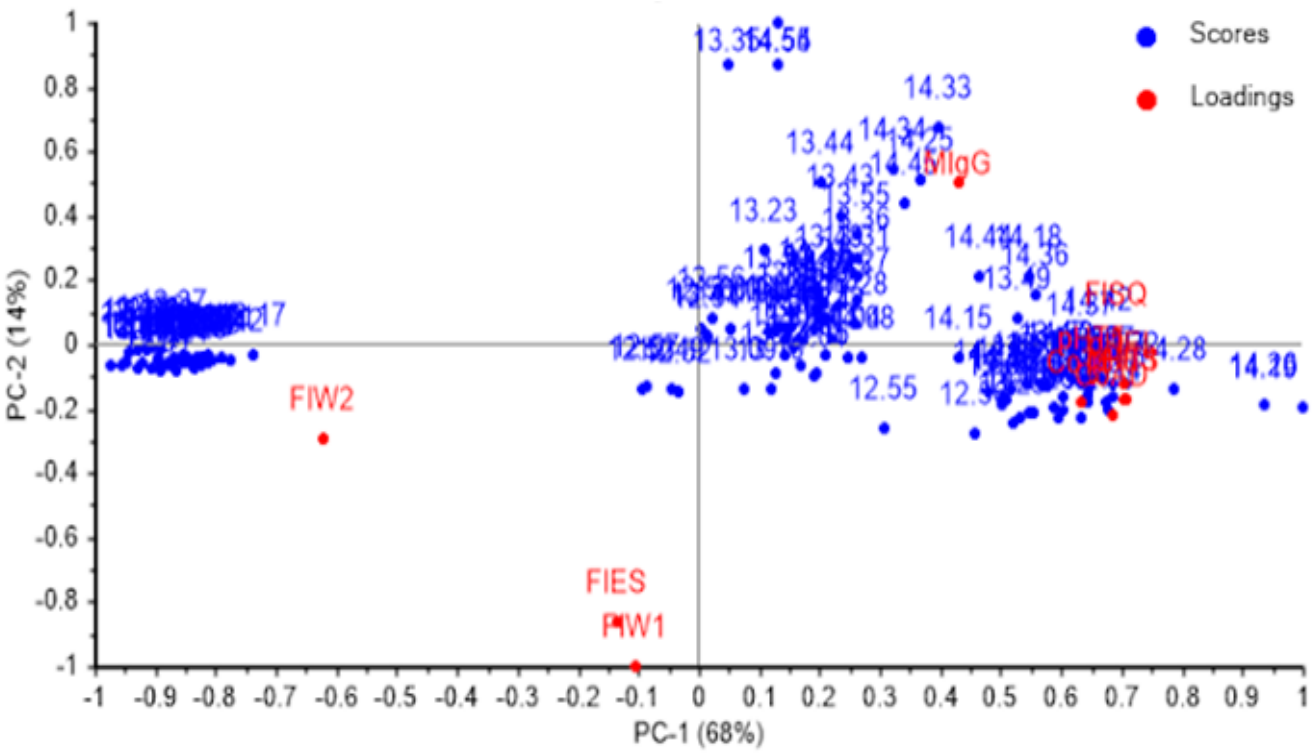

Figure 5. Bi-plot of parameter loadings and scores graph.

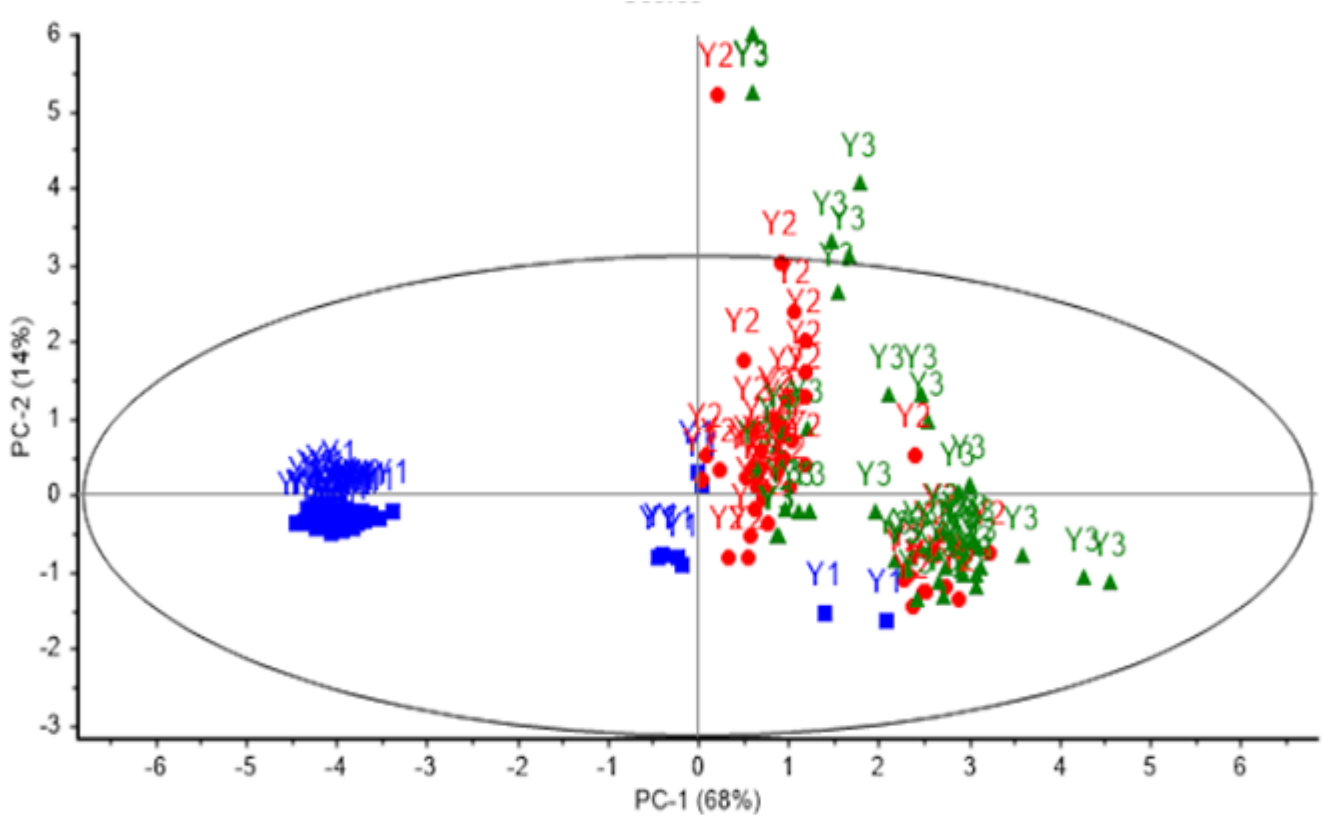

Figure 6. Score's graph showing annual campaigns batch patterns.

Table 5. Cluster analysis summary of batch patterns in score graph (equal color stand for the same class)

Year 1 campaign				Year 2 campaign				Year 3 campaign			
Batch	Class	Batch	Class	Batch	Class	Batch	Class	Batch	Class	Batch	Class
12.01	2	12.31	2	13.01	0	13.31	0	14.01	0	14.34	1
12.02	2	12.32	2	13.02	0	13.32	1	14.02	0	14.35	1
12.05	2	12.33	2	13.03	0	13.33	0	14.03	1	14.36	1
12.07	2	12.34	2	13.04	0	13.34	1	14.04	1	14.37	1
12.09	2	12.36	2	13.05	1	13.35	1	14.05	0	14.38	1
12.10	2	12.37	2	13.06	0	13.36	0	14.06	1	14.39	0
12.11	2	12.38	2	13.07	0	13.37	0	14.07	1	14.40	0
12.12	2	12.39	2	13.08	0	13.38	0	14.08	1	14.41	0
12.13	2	12.40	2	13.09	0	13.39	0	14.10	0	14.42	1
12.14	2	12.41	2	13.10	0	13.40	1	14.12	0	14.43	1
12.15	2	12.42	2	13.11	0	13.41	2	14.13	0	14.44	1
12.16	2	12.43	2	13.12	0	13.42	1	14.14	0	14.45	1
12.17	2	12.44	2	13.13	0	13.43	1	14.15	0	14.46	1
12.18	2	12.45	2	13.18	0	13.44	1	14.16	0	14.47	0
12.19	2	12.46	2	13.19	0	13.45	0	14.17	0	14.48	0
12.20	2	12.47	2	13.20	0	13.46	0	14.18	1	14.49	0
12.21	2	12.48	2	13.21	0	13.47	1	14.19	1	14.50	1
12.22	2	12.49	0	13.22	1	13.48	1	14.24	0	14.51	1
12.23	2	12.50	0	13.23	0	13.49	1	14.25	1	14.52	1
12.24	2	12.52	0	13.24	0	13.50	0	14.26	1	14.53	1
12.25	2	12.53	0	13.25	0	13.53	0	14.27	0	14.54	1
12.26	2	12.54	0	13.26	0	13.54	0	14.28	1	14.55	1
12.27	2	12.55	0	13.27	0	13.55	0	14.30	1	14.56	0
12.28	2	12.56	0	13.28	0	13.56	2	14.31	1	14.57	1
12.30	2	12.57	0	13.29	0			14.33	1		

first confrontation to IgG capture and anion exchange new technologies at that time, as well as the technical challenges for proper implementation at manufacturing scale.

Also from figure 6, for the subsequent campaigns of second and third years the situation is far different as MIgG production gradually increased, although with fluctuations inherent to cell culture perfusion mode [25]. According to cluster analysis (see also Table 5) there are two more classes or group of batches at the right side that belong indistinctly to Y2 and Y3 campaigns, but difficult to distinguish in those patterns which parameters have more impact. This due to MIgG or buffer solution parameters with their intrinsic variability due to manual preparation, or even both, taking into account at this point that FlSQ has been defined more accurately since the end of first campaign.

In view of the above, the SIMCA method was applied in order to confirm differences among campaigns. From Coomans' graph shown in figure 7, it can be corroborated the particular behavior of campaign Y1 as formerly explained, being identified in two separated group by the green dots.

It can also be verified that campaigns Y2 and Y3 are similar regarding the operational praxis, except some Y2 middle of campaign batches 13.26 to 13.42 identified by blue dots at the upper left quadrant, including an isolated end of the year batch 13.56. This is due to eventual composition changes of second wash buffer solution affecting pHT5, which was consequently reflected in protein A column eluate pHEL with some increase. Coincidently, MIgG fluctuations entering the process in that period made more complicated the harvest volume pooling operations, and then FlES was adjusted from batch to batch under technical considerations in this particular case.

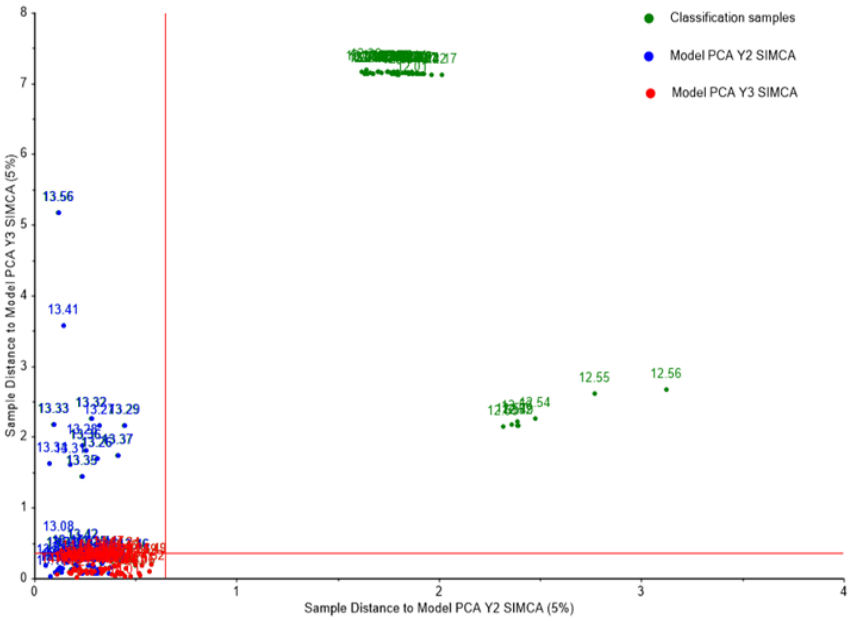

Figure 7. Coomans' graph resulting from SIMCA method application for comparing campaigns.

Continued process verification planning

In addition to the above results, process knowledge and experience were brought again in order to elaborate a sound CPV monitoring plan. In essence, parameters with major influence on process variability should have a weekly follow up, while those with less incidences, its trending can be analyzed monthly, or quarterly in the case of KOP, although technological and operational factors could also define such fre-

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quency [9]. In the case of correlated parameters, their monitoring weekly or monthly depends on their pertinence to reflect related unit operation actual state, for instance pHEL prevails over pHES in depicting the elution step status under such criterion.

Regarding flows applied to chromatographic steps, it was decided to keep them in a fixed value from batch to batch with a defined tolerance, based on data processing results that cover actual MIgG inlet variability, and they should also be monitored weekly or monthly according to their relevance in related unit operation. The conceived plan is summarized in Table 6.

Even when in practice it has been successful so far in obtaining reliable information on process actual state of control and the excursions detection from normal operational conditions, it is advisable to carry out periodic reviews through risk analysis reissue. This is relevant to face possible process changes, as well as the analysis of data collected in the period for keeping the CPV plan updated.

Table 6. Summary of conceived monitoring plan for continued process verification

Parameter	Category	Measurement feature	Proposed trending frequency
pHEL	CPP	Online from automatic control	Weekly from batch-to-batch data
CoSQ	CPP	Inline	Monthly from batch-to-batch data
FISQ	CPP	Inline	Monthly from batch-to-batch data
pHES	CPP	Offline on site	Monthly from batch-to-batch data
TINV	CPP	Offline on site	Monthly from batch-to-batch data
FIES	CPP	Online from automatic control	Weekly from batch-to-batch data
MIgG	CPP	Offline at lab	Weekly from batch-to-batch data
pHT5	CPP	Offline on site	Weekly from batch-to-batch data
CoT5	CPP	Offline on site	Monthly from batch-to-batch data
FIW2	CPP	Online from automatic control	Monthly from batch-to-batch data
FIW1	CPP	Online from automatic control	Monthly from batch-to-batch data
CoAD	CPP	Offline on site	Weekly from batch-to-batch data
pHT1	CPP	Offline on site	Monthly from batch-to-batch data
CoEQ	KOP	Offline on site	Quarterly from at random selected batches data
FIEQ	KOP	Inline	Quarterly from at random selected batches data
FIW3	KOP	Online from automatic control	Quarterly from at random selected batches data
FITE	KOP	Online from automatic control	Quarterly from at random selected batches data
MPAE	KOP	Offline at lab	Quarterly from at random selected batches data

Conclusions

The applied approach in the present work was effective in determining critical process parameters and inter-batch variability patterns in order to conceive a rational monitoring plan for continuous process verification under scientific basis, as well as the possibility to disclose eventual process changes. Such features were congruently corroborated with the information from the operational incidences log of examined production campaigns corresponding to the downstream first purification process under study. This leads to a more robust and controllable process, which can be traced back. Moreover, the inclusion of new steps and their modifications could be classified and followed by this methodology.

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Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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