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# A validated capillary electrophoresis method to check for batch-to-batch consistency during recombinant human glycosylated interleukin-7 production campaigns

Youssef Alahmad [a], Myriam Taverna [a], Hanane Mobdi [b], Jérémy Duboeuf [b], Anne Grégoire [b], Iann Rancé [b], Nguyet Thuy Tran [a,*]

[a] Univ Paris-Sud, Laboratoire de Protéines et Nanotechnologies en Sciences Séparatives (JE 2495), Faculté de Pharmacie, 92296 Châtenay-Malabry, France
[b] Cytheris, Technopolis, 175 rue Jean-Jacques Rousseau, 92130 Issy-les-Moulineaux, France

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ABSTRACT

This work reports the validation of a simple CZE method to be used in quality control of recombinant human glycosylated interleukin-7 (rhIL-7) batches produced in Chinese Hamster Ovary (CHO) cells. The separation buffer was a 25 mM sodium borate at pH 10 containing 12 mM diaminobutane (DAB) used as a dynamic coating agent of the capillary. This method allowed the separation of seven peaks ranging from low to high sialylated glycoforms. An extensive study on conditioning methods of the capillary has been conducted to yield repeatable results. Excellent RSD of EOF mobility (less than 0.6%) was obtained when conditioning included capillary equilibration under virtual analyses and storage in 0.1 M NaOH overnight. Method specificity has been demonstrated to be able to discriminate different rhIL-7 glycoforms produced in CHO from formulation matrix. Linearity was demonstrated between 0.5 and 4 mg/mL. LOQ was 0.5 mg/mL. Repeatability (RSD < 1.4 and 3.3% for $t_m$ and *A%*, respectively), intermediate precision of inter-day (RSD < 2.1 and 4.5), inter-analyst (RSD < 2.0 and 3.0) and inter-equipment (RSD < 3.8 and 3.7 for electrophoretic mobility and *A%*, respectively) were all very satisfactory. Evaluation of robustness revealed that pH and DAB concentration are critical parameters in the method while slight alteration of ionic strength of electrolyte or change of capillary source did not affect the results. Finally the method was shown to provide reliable informations to address comparability studies and batch-to-batch consistency of biomanufactured rhIL-7.



## 1. Introduction

Interleukin-7 (IL-7) is secreted by lymphoid tissues and belongs to the cytokine family. It has a well-known role in B- and T-cell lymphopoiesis [1]. Human IL-7 is a 32–35 kDa glycoprotein containing three potential N-glycosylation and one potential O-glycosylation sites that contribute to its stability, *in vivo* half-life and mean residence time [2]. Considering its significant clinical and biomedical potential in immunotherapy, several expression systems have been exploited for the production of recombinant human IL-7 (rhIL-7).

Glycosylated rhIL-7 produced from Chinese Hamster Ovary (CHO) cells is currently under clinical development since no therapeutic rhIL-7 is yet commercially available.

According to the host system, glycosylation profile will vary but glycoform complexity of the purified product may also be dependent on several other factors, including purification process and manufacturing procedures. Thus, a robust analytical method is required to control this microheterogeneity and monitor its consistency from lot-to-lot. In addition, the validation characteristics should follow guidelines and recommendations of the International Conference on Harmonization (ICH) [3–6].

Due to their robustness and reproducibility, chromatography-based analytical methods are often preferred over electrophoretic ones for quality control (QC). Nevertheless, they are not always able to resolve all protein variants/isoforms/glycoforms [7]. In this respect, capillary electrophoresis (CE) is regarded as one of the best alternative technique and especially for analysing biotechnology-derived therapeutic glycoproteins [8,9]. A number of reviews have reported strategies available to monitor glycoform heterogeneity by CE [10–14]. Only a few of them have focused on validation issues

*Abbreviations:* BGE, background electrolyte; CE, capillary electrophoresis; CHO, Chinese Hamster Ovary; CZE, capillary zone electrophoresis; DAB, diaminobutane; *E. coli*, *Escherichia coli*; EOF, electroosmotic flow; Eu. Ph., European pharmacopoeia; ICH, International Conference on Harmonization; IL-7, human interleukin-7; QC, quality control; rhEPO, recombinant human erythropoietin; rhIL-7, recombinant human interleukin-7.

* Corresponding author. Tel.: +33 1 46 83 59 03; fax: +33 1 46 83 59 44.
*E-mail address:* thuy.tran-maignan@u-psud.fr (N.T. Tran).

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of CE methods devoted to protein analysis in a QC environment [15,16]. Validated CE methods for isoform separation of recombinant glycoproteins rely mainly on three CE modes: capillary zone electrophoresis (CZE) [17,18], capillary isoelectrofocusing (CIEF) [18,19] and sodium dodecyl sulfate-capillary gel electrophoresis (SDS-CGE) [20,21].

When dealing with glycoprotein analysis by CE, the two main challenges are first, to reduce wall adsorption of proteins by permanent or dynamic modification of the capillary [22,23] and second, to ensure a stable electroosmotic flow (EOF), which is achieved by a good control of the surface state of the capillary. Both these features impact directly on repeatability, reproducibility and separation performances of the method. In a QC context and due to unknown long-term stability of permanent coating, dynamic coatings are often preferred. To achieve efficient and reproducible dynamic coating, a particular attention has to be paid on capillary conditioning [24] since the surface treatment influences the EOF mobility and consequently migration time and peak areas of the glycoforms [16,25]. In a recent work, Gómez and Sandoval [26] have compared different capillary conditionings with strong base or acids, and showed that the best precision was provided by a sequential NaOH–HCl treatment. The same authors emphasized the role of extensive rinses with the background elycetrolyte (BGE) at high flow rates to reach relatively rapid equilibration of the capillary once it is properly conditioned. In other studies, capillary equilibration has also been achieved either by voltage application during long time periods (12 h) for recombinant human erythropoietin (rhEPO) [27], or by several preliminary short runs of neutral marker as reported for the vascular endothelial growth factor [28]. From the different works, it appears that conditioning of the capillaries as well as rinsing method employed between runs are at least as important as the separation method itself to end up with a routine QC method in CE.

rhIL-7 employed in this validation study was produced in a CHO cell-based expression system. Extensive glycan analyses on purified rhIL-7 revealed typical CHO complex-type sialylated N-glycans (unpublished data). We recently reported the first separation of rhIL-7 glycoforms by CE under extremely acidic conditions [29]. Although the CE profile of rhIL-7 obtained was able to give information on subtle modifications of rhIL-7 glycosylation between batches, it was too complex to validate the method and to be used in a QC reviewed production area. Thus, this developed method was considered as a supportive analytical tool to monitor process and scale up improvements.

The aim of the present work was to develop and to validate a new CZE method, combining alkaline conditions and diaminobutane (DAB) as additive in BGE to profile the different rhIL-7 glycoforms. In order to provide a reproducible method to be used in QC, different capillary conditioning methods were compared. Finally, the method was applied to profile non-glycosylated rhIL-7 from *Escherichia coli* (*E. coli*) and to control protein production under current good manufacturing practices (cGMP) and lot-to-lot consistency.

## 2. Materials and methods

### 2.1. Production of rhIL-7

rhIL-7 was produced from CHO cell cultures by Cytheris S.A. (Issy Les Moulineaux, France). Purified rhIL-7 batches were obtained according to a well defined purification process involving three consecutive chromatographic steps: anion-exchange chromatography, cation-exchange chromatography and hydrophobic interaction chromatography. Prior to product release, the protein was formulated in 10 mM sodium acetate buffer containing 100 mM of NaCl at pH 5 and stored at −20 °C. Three batches (A, B and C) have been used in this study. Batches B and C differ from batch A by an improved, more selective purification process. Batch C differs from batch B by a strict application of cGMP. Batch comparison was conducted using the optimized CZE method. Non-glycosylated rhIL-7 was also obtained from Cytheris S.A. after production from *E. coli* culture.

### 2.2. Reagents

1 M NaOH, 1 M HCl and thiourea were obtained from (ProLabo, Fontenay-sous-Bois, France). Boric acid, urea, HEPES, tricine, Tris, CHES, CAPS, NaCl and DAB were purchased from Sigma (St. Louis, MO, USA). All buffers were prepared with Milli-Q water using a Direct-Q® 3 UV purification system from Millipore (Bedford, MA, USA) and were filtered through a 0.2 μm Millex membrane (Millipore, Bedford, MA, USA). Buffers were prepared at fixed ionic strength by mixing determined volumes of 1 M stock solutions of acidic and alkaline species according to the protocol provided by Phoebus software (Analis, Suarlée, Belgium). A pH meter (WTW GmbH, Weilheim, Germany) was used to check the pH of the buffers.

### 2.3. Sample preparation

Prior to the CZE analyses, samples were filtered by ultracentrifugation (cut off membrane of 3 kDa) using Nanosep centrifugal devices (VWR, Fontenay-sous-Bois, France) adjusting their concentration to 2 mg/mL. After filtration, the samples were divided into aliquots of 5 μL and stored at −20 °C.

### 2.4. CE

#### 2.4.1. Apparatus

Experiments were performed using mainly a P/ACE 5500 system equipped with a UV-detector (Beckman Coulter, Fullerton, CA, USA). Two other instruments from the same manufacturer: P/ACE MDQ and PA 800 systems were also employed to evaluate the inter-equipment repeatability. Most of the time, fused silica capillaries (Phymep, Paris, France) were employed with an effective length of 40 cm (total length of 47 cm for the P/ACE 5500 system and 50 cm for the other systems) × 50 μm ID and 375 μm OD. For the robustness study and in order to compare results obtained from different sources or batches of capillaries, capillaries were also purchased from Beckman Coulter (Fullerton, CA, USA).

#### 2.4.2. CZE method

Classical conditioning: each new capillary was first conditioned with the following sequence of rinses: water for 5 min, 1 M NaOH for 5 min, 0.1 M NaOH for 5 min, water for 5 min.

Optimized conditioning: the classical conditioning was followed by five "virtual analyses" which were done using the following method: rinses with water for 2 min, 0.1 M NaOH for 2 min, water for 2 min, BGE for 2 min; injection of 0.1 mg/mL of thiourea for 5 s (for EOF measurements), separation under 20 kV or 21.2 kV (for P/ACE5500 and MDQ, respectively) during 30 min, rinses with water for 3 min. After these 5 analyses, the capillary was rinsed with 0.1 M NaOH for 3 min and the capillary ends were immerged in 0.1 M NaOH overnight.

Separation conditions: the BGE was a 25 mM borate buffer, pH 10, containing 12 mM DAB. It was prepared as follows: 100 mL of stock solution of 0.5 M boric acid was prepared by dissolving 3.091 g of boric acid (>99.5%) in 100 mL of water. This solution was stored at +4 °C not more than 2 weeks. To prepare 50 mL of buffer at fixed ionic strength of 25 mM and pH 10, 2.85 mL of stock solution of boric acid were mixed with 1.25 mL of 1 M NaOH, then water was added to a total volume of 50 mL and the pH was checked. Finally, 96.5 mg

of DAB provided as an HCl salt (>98%) was added to the buffer. Each vial buffer was changed after each analysis. Prior to its first use, the capillary was washed with water for 5 min. rhIL-7 analyses were achieved using the method called "virtual analysis" in the optimized conditioning described above and by injecting 2 mg/mL of rhIL-7 instead of thiourea. All rinses were performed under 20 psi, while samples were introduced into the capillary by hydrodynamic injection for 5 s under 0.5 psi. The capillary was thermostated at 25 °C. UV detection was performed at the cathode end at 214 nm. Conditioning optimization was carried out using batch A.

The relative area for one peak (i) was calculated using the following equation:

$$A_i\% = \frac{A_i}{\sum_{i=1}^{7} A_i}$$

where $A$ is the absolute peak area of peak, and the $\sum A$ is the sum of areas of the seven peaks of interest.

### 2.5. Methodology of the validation

All validation procedures were carried out using batch B. Four peaks (peak 2–5) in the rhIL-7 profile were considered during the method validation. Filtered formulation buffer was used for the specificity test. Based on 10:1 ratio of S/N (signal to noise), LOQ was determined by injecting the placebo or blank samples (filtered buffer of formulation) and measuring the signal over 10 min of the baseline in the migration time region where rhIL-7 peaks are supposed to migrate. The linearity of the method was studied between 0.5 and 4 mg/mL, five concentrations were chosen (0.5, 1, 2, 3 and 4 mg/mL) and three analyses were performed at each concentration level. Statistical exploitation of the data included Cochran test to check variance homogeneity and ANOVA test to validate the linear model. The precision of the method was investigated at different levels as follows. For intra- and inter-day repeatability, the assays were repeated six times within 1 day (intra-day) and once per day over a 5-day period (inter-day). Inter-analyst repeatability was conducted by three analysts; each one has carried out one analysis by applying the same method in the same day. Inter-equipment repeatability was evaluated by applying the same method using three different apparatus (P/ACE 5500, MDQ and PA 800), and one analysis was run on each apparatus. Robustness was evaluated over four parameters: BGE pH, concentration of the dynamic coating agent (DAB) in the BGE, ionic strength of buffer and capillary source. rhIL7 was therefore performed under BGE pH at 9.8, 10 or 10.2; or using DAB concentrations of 11, 12 or 14 mM; ionic strength of 25 or 30 mM and finally on three different capillaries from different suppliers or batches. For each parameter tested, the analysis was repeated three times in the same day.

### 2.6. Applications of the method

Non-glycosylated rhIL-7 from *E. coli* was analysed using the validated method developed. The method was also applied to compare rhIL-7 produced from slightly different purification processes (batch A *vs.* batch B). The consistency of product quality was evaluated by a comparison of batch B *vs.* batch C differing by the strict application of cGMP in the case of batch C.

## 3. Results and discussion

### 3.1. Optimization of the method

In a previous work [29] we reported acidic conditions for the separation of rhIL-7 glycoforms expressed in CHO cells. Since these conditions did not allow the method validation due to a too complex profile, we have tried to optimize the glycoforms separation of purified rhIL-7 in alkaline conditions (pH 8–10). Several BGE (tricine, Tris, CAPS, CHES, HEPES, phosphate and borate) were tested. The borate was retained as the best BGE. However, the adsorption being still important, the DAB was added in order to prevent adsorption onto the capillary surface. The DAB was tested between 2 and 20 mM, and 12 mM was the optimal concentration. Then, we have carried out exhaustive attempts to improve the resolution by varying the voltage, the capillary length, the sample media (salts, pH) and finally by the use of chaotropic agents (7 M urea). The better resolution obtained led to 7 distinct peaks that were not fully resolved but leading to a profile compatible with proper integration of reproducible peaks.

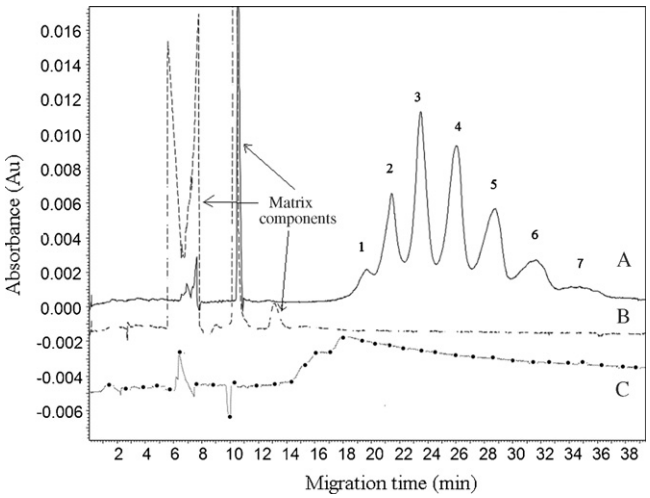


**Fig. 1.** CZE profile of rhIL-7 produced in CHO cells (solid line) (A), sample matrix (dashed line) (B) and rhIL-7 produced in *E. coli* (dotted line) (C). Conditions: 25 mM sodium borate at pH 10, 12 mM DAB, applied voltage: 20 kV, detection: 214 nm, fused silica capillary (40 cm × 50 μm), rhIL-7 at 2 mg/mL. Peaks 2–5 are the main peaks selected for the optimization of the capillary conditioning.

### 3.2. Optimization of the capillary conditioning

The best separation of rhIL-7 glycoforms, allowing quantitative data to be deduced, was achieved by CZE using a dynamic coating with DAB. The optimal BGE was a 25 mM sodium borate buffer at pH 10 containing 12 mM of DAB. The corresponding profile shows seven main peaks ranging from low to high sialylated glycoforms (Fig. 1A), the two peaks observed before the glycoform profile represent components of the sample matrix (Fig. 1B). An extensive study of the effect of capillary conditioning methods (Table 1) on the repeatability of the results was conducted. Five conditioning methods were tested using a new capillary for each method. Four peaks (peaks 2–5) of rhIL-7 were considered for this study. The evaluated parameters were: the value and RSD of EOF mobility (Fig. 2), RSD of migration times ($t_m$) and relative peak areas ($A$%) (Table 1). As shown in Fig. 2, EOF value depends on the concentration of DAB in BGE: the higher is the DAB concentration (methods A and B), the lower is the EOF value. RSD of EOF mobility is better when conditioning methods include storage of capillary in 0.1 M NaOH for 2 h (method D *vs.* method A). Results show that the higher is the number of virtual analyses, the better is this repeatability (methods B and C). It seems that the virtual analyses included in conditioning (method E *vs.* method D) contribute to the better repeatability in term of the mobility electroosmotic $\mu_{eo}$ and $t_m$ observed. This can be explained by a more efficient fixation of DAB onto the silica surface. Finally, satisfactory RSD of EOF mobility, and consequently of $t_m$ and $A$% was achieved with methods B and C which both include a capillary equilibration under five virtual analyses followed by a

**Table 1**
Comparison of different capillary conditioning methods on the RSD ($n = 5$) of migration time $t_m$ and relative peak area $A$%. The RSD values represent a range from the smallest to the highest values obtained for the four peaks.

| Method | Conditioning cycles | DAB concentration in BGE[a] (mM) | RSD% of $t_m$ | RSD% of $A$% |
|---|---|---|---|---|
| A | Classical conditioning | 14 | 11.0–19.6 | 4.2–49.2 |
| B | Classical conditioning, 5 virtual analyses, storage under 0.1 M NaOH overnight | 14 | 1.3–3.4 | 0.1–4.1 |
| C | Classical conditioning, 5 virtual analyses, storage under 0.1 M NaOH overnight | 12 | 1.2–1.9 | 0.7–2.7 |
| D | Classical conditioning, storage under 0.1 M NaOH for 2 h | 12 | 4.8–8.5 | 1.9–12.8 |
| E | Classical conditioning, 1 virtual analysis, storage under 0.1 M NaOH for 2 h | 12 | 2.9–4.0 | 1.4–13.4 |

[a] BGE; 25 mM sodium borate at pH 10.

storage of the capillary in 0.1 M NaOH overnight. A similar conditioning method using a lower concentration of DAB was previously reported in our laboratory for peptide separation [24]. In our case, the "static" mode of conditioning by the storage of the capillary filled with 0.1 M NaOH overnight may lead to the same effect as it has been done for the capillary etching with HF [26]. Moreover, we can hypothesize that equilibration of the capillary, once it is properly conditioned, can be achieved either by running analyses of EOF marker for example, or by applying voltage [24] as in the Eu. Ph. method for the analysis of rhEPO [27].

### 3.3. Method validation

All the tests carried out to validate the method have been performed on one single batch B of rhIL-7 produced from CHO cells.

#### 3.3.1. Specificity

As defined by ICH guidelines [6], the specificity of an analytical method is its ability to discriminate unequivocally the analyte from closely related components that might be present (impurities, matrix). The specificity of the CZE method developed was demonstrated by analysing the storage buffer of rhIL-7. The profile obtained from the sample matrix is markedly different from the rhIL-7 one (Fig. 1B). Indeed, the sample matrix does not produce peaks in the migration time region of rhIL-7 glycoforms (Fig. 1A). In conclusion, the specificity of this method is assessed.

#### 3.3.2. LOQ

The limit of quantification (LOQ) is defined as the lowest analyte amount, which can be quantified with acceptable precision and accuracy [6]. Based on S/N ratio of 10, the LOQ estimated for the peak 2 (the smallest among the four main peaks considered for the quantification of the rhIL-7) was 0.5 mg/mL.

#### 3.3.3. Linearity

For each of the five concentrations investigated, three injections were performed and the absolute areas were plotted against the concentration. The linearity was evaluated for the main peaks 2–5 (Table 2). The determination coefficient $R^2$ ranged from 0.996 to 0.998. On a significance level of 0.05, all observed values (obtained with the Cochran test) were lower than the critical value demonstrating the variance homogeneity at each concentration. Then, ANOVA test on the adjusted and residual variances was performed, and results demonstrated that the linear regression fits the experimental data for concentrations ranging from 0.5 to 4 mg/mL.

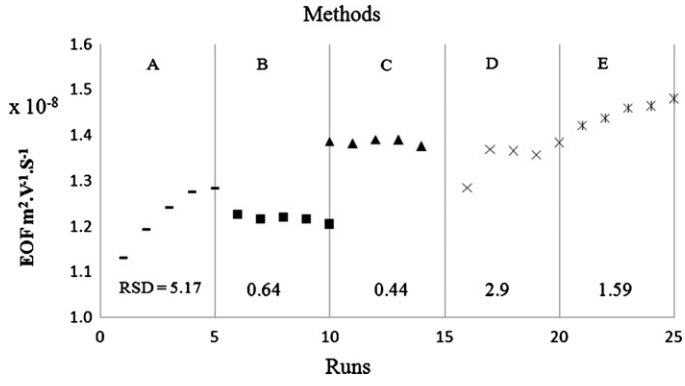


**Fig. 2.** Comparison of different capillary conditioning methods on the EOF value and the corresponding RSD ($n = 5$). A, B, C, D and E methods are detailed in Table 1.

#### 3.3.4. Precision

The precision is defined as the degree of scatter between a series of results obtained under identical condition [6]. Using the separation and conditioning optimized previously, intra- and inter-day as well as inter-analyst and inter-equipment reproducibilities were evaluated taking into account both $t_m$ and $A$% for the four main peaks (peaks 2–5). Excellent intra-day and inter-day repeatabilities were obtained for $t_m$ (RSD < 1.4% and 2.1%, respectively) probably thanks to the high stability of EOF mobility resulting from the optimized conditioning and dynamic coating of the capillary methods (Table 3). Satisfactory intra- and inter-day repeatabilities were also observed for the $A$% (RSD less than 3.3% and 4.5%, respectively) (Table 3).

The results have shown a good inter-analyst repeatability with RSD% < 2.0% for $t_m$ and RSD% < 3.0% for $A$% (Table 4).

Finally, for inter-equipment repeatability, RSD of the electrophoretic mobility ($\mu_{ep}$) has been calculated instead of migration time since the total length of capillary between the three apparatus is different. RSD were lower than 3.8% for both $\mu_{ep}$ and $A$% (Table 4).

#### 3.3.5. Robustness

Robustness of an analytical procedure determines its ability to remain unaffected by small variations in method parameters. It

**Table 2**
Data of linearity study.

| Peak | Regression equation | $R^2$ |
|---|---|---|
| 2 | $y = 231786x - 32189$ | 0.996 |
| 3 | $y = 446290x - 60483$ | 0.997 |
| 4 | $y = 446641x - 508$ | 0.998 |
| 5 | $y = 351710x - 10242$ | 0.998 |

**Table 3**
Data of intra- and inter-day repeatabilities.

| Peak number | Intra-day | | | | Inter-day | | | |
|---|---|---|---|---|---|---|---|---|
| | $t_m$ (min) | | $A$% | | $t_m$ (min) | | $A$% | |
| | Mean | RSD[a] | Mean | RSD[a] | Mean | RSD[a] | Mean | RSD[a] |
| 2 | 20.4 | 1.2 | 15.1 | 2.2 | 21.1 | 1.5 | 12.7 | 4.3 |
| 3 | 22.3 | 1.1 | 23.7 | 2.0 | 23.2 | 1.6 | 24.6 | 3.0 |
| 4 | 24.6 | 1.3 | 24.3 | 1.3 | 25.6 | 1.9 | 24.6 | 3.4 |
| 5 | 26.9 | 1.4 | 18.0 | 3.3 | 28.2 | 2.1 | 18.3 | 4.5 |

[a] RSD were calculated from $n = 6$ and $n = 5$ analyses for intra- and inter-day, respectively.

**Table 4**
Data of inter-analyst and inter-equipment repeatabilities.

| Peak number | Inter-analyst | | | | Inter-equipment | | | |
|---|---|---|---|---|---|---|---|---|
| | $t_m$ (min) | | A% | | $\mu_{ep}$ ($m^2 v^{-1} s^{-1}$) | | A% | |
| | Mean | RSD[a] | Mean | RSD[a] | Mean ($\times 10^{-9}$) | RSD[a] | Mean | RSD[a] |
| 2 | 21.0 | 1.9 | 12.6 | 3.0 | 8.1 | 3.8 | 15.1 | 3.3 |
| 3 | 23.0 | 1.8 | 24.9 | 1.0 | 8.7 | 3.7 | 24.2 | 3.7 |
| 4 | 25.4 | 2.0 | 24.7 | 3.0 | 9.3 | 3.7 | 25.0 | 1.3 |
| 5 | 27.9 | 2.0 | 19.2 | 2.0 | 9.9 | 3.5 | 18.4 | 1.1 |

[a] RSD were calculated from $n = 3$ analyses.

is mandatory to evaluate the validation characteristics to identify which are the sensitive parameters of the method, especially when considering the possibility of method transfer between different laboratories and equipments. Robustness was therefore evaluated through varying the pH, DAB concentration and ionic strength of BGE around their optima obtained during the method optimization. Capillaries from different suppliers (capillaries 1 and 2 from Phymep and 3 from Beckman Coulter) or different batches from the same supplier (capillaries 1 and 2) were also tested. Means and RSD for EOF mobilities, $t_m$ and A% of the four selected peaks have been calculated. Parameter was considered "not robust" as soon as statistical results showed significant differences in either $t_m$ or A% and this for one of the four main peaks (peaks 2–5) investigated. For instance, results obtained for peak 4 for all the experimental parameters investigated are summarized in Table 5. From the overall results, pH is a critical parameter that affects the method since even if variance between the samples were homogenous, significant difference between the means was observed for several peaks. Student test at 95% CI showed also significant differences between $t_m$ and A% obtained under the different DAB concentrations. In conclusion, pH and DAB concentration parameters should be adjusted with great accuracy because of their key role on EOF mobility and consequently on migration times which can impact on peak assignment. The same statistical tests revealed that variation of ionic strength of BGE or capillary sources did not affect the method results (neither quantitatively nor qualitatively).

### 3.3.6. System suitability

The appropriate performance of the analytical system is generally assessed by a suitability test, which aims at establishing critical limits for selected key parameters. The CZE system was considered properly equilibrated and conditioned when the baseline remained stable within the first three analyses. In addition, the RSD of EOF mobility has to be less than 2% over these three analyses. Seven peaks corresponding to sialylated glycoforms (1–7) have to be clearly detected and should migrate between 16 and 40 min (Fig. 1A). The migration time of each peak has to be within ranges specified in Table 5. The resolution of the critical peak pair (peak 3–peak 4) has to be higher than 1. This critical value does not correspond however to a full resolution. The proportion of the different peaks has to be quantitatively similar to the percentage content expected for each rhIL-7 glycoform within limit ranges indicated in Table 6.

**Table 5**
Robustness evaluation concerning pH, ionic strength, DAB concentration in the BGE and capillary source, data for peak 4.

| Separation condition | $\mu_{eo}$ ($m^2 v^{-1} s^{-1}$) | | $t_m$ (min) | | A% | |
|---|---|---|---|---|---|---|
| | Mean ($\times 10^{-8}$) | RSD%[a] | Mean | RSD%[a] | Mean | RSD%[a] |
| pH | | | | | | |
| 9.8 | 1.5 | 0.2 | 25.2 | 0.5 | 24.5 | 0.7 |
| 10 | 1.6 | 0.4 | 24.8 | 1.0 | 24.2 | 2.6 |
| 10.2 | 1.6 | 0.2 | 23.6 | 0.9 | 24.3 | 2.0 |
| Ionic strength (mM) | | | | | | |
| 25 | 1.6 | 0.3 | 23.6 | 0.6 | 24.0 | 1.3 |
| 30 | 1.6 | 1.8 | 22.4 | 3.5 | 24.3 | 2.4 |
| DAB concentration (mM) | | | | | | |
| 11 | 1.6 | 0.1 | 22.2 | 0.3 | 23.5 | 0.1 |
| 12 | 1.6 | 0.5 | 24.3 | 0.7 | 24.8 | 0.9 |
| 14 | 1.4 | 0.6 | 30.2 | 0.6 | 26.4 | 1.6 |
| Capillary | | | | | | |
| 1 | 1.5 | 0.8 | 24.1 | 1.0 | 24.8 | 0.8 |
| 2 | 1.6 | 0.5 | 22.6 | 1.5 | 24.4 | 0.5 |
| 3 | 1.6 | 0.4 | 23.4 | 1.2 | 24.5 | 1.4 |

[a] RSD was calculated from $n = 3$ analyses.

### 3.4. Applications

#### 3.4.1. Analysis of rhIL-7 from E. coli

As a first application, the non-glycosylated rhIL-7 produced from *E. coli* [8] (Fig. 1C), was analysed using the validated CZE method. The profile of the *E. coli* rhIL-7 showed non-resolved peaks migrating earlier than the least sialylated glycoform of rhIL-7 from CHO cells (Fig. 1A). This corroborates the dependency of the migration on the glycan content as well as sialylation degree of a protein using CZE. Also, an important adsorption phenomenon was observed for rhIL-7 expressed in *E. coli* as demonstrated by the tailing peak. This may be explained by the absence of glycan that usually screen the hydrophobic domains and contribute thereby, to avoid adsorption of rhIL-7 on the capillary wall.

#### 3.4.2. rhIL-7 from different purification process

The validated method was also employed to compare two rhIL-7 samples (batches A and B) purified from CHO, differing by the glycoform selectivity during the purification process (Fig. 3A). As compared to batch B, first migrating glycoforms of batch A (peaks 1–3) (Fig. 3A) showed an increase in peak intensity linked to a decrease of peak intensity of the last migrating ones. These quantitative differences in the migration profiles reveal the high glycosylation quality of batch B *vs.* batch A. Indeed, downstream purification for batch B, involved a new anion-exchange chromatographic media that is more selective towards rhIL-7 glycoforms

**Table 6**
Percentage content of each rhIL-7 glycoform (in term of relative peak area) and their migration time.

| Glycoforms | Percentage content of peak area | Migration time (min) |
|---|---|---|
| 1 | 0–15 | 17–20 |
| 2 | 10–25 | 18–22 |
| 3 | 15–30 | 20–25 |
| 4 | 15–30 | 22–27 |
| 5 | 10–30 | 25–29 |
| 6 | 5–20 | 25–33 |
| 7 | 0–15 | 27–36 |

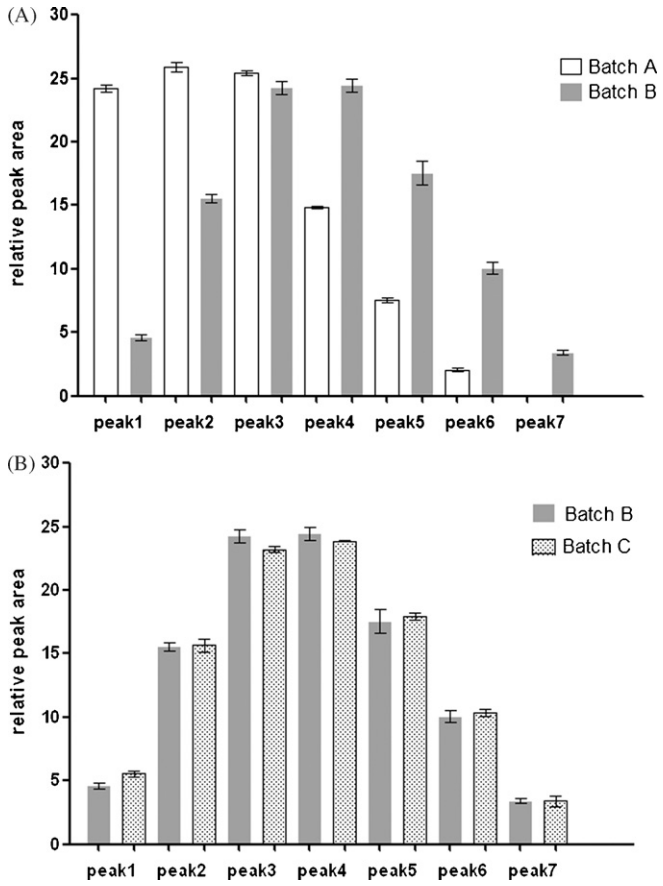


**Fig. 3.** Comparison of glycoform pattern by CZE of three different rhIL-7 batches. (A) Comparison between batches A and B, (B) comparison between batches B and C. Batch A: product purified via standard process, batch B: pilot production purified using an optimized and more selective purification process, and batch C: cGMP manufacturing. Values represent the means ± SD (from three consecutive analyses). Conditions are identical to those in Fig. 1.

and CHO impurities, therefore yielding a more glycosylated and sialylated final product than that of batch A. Since *in vivo* activity parameter of rhIL-7 such as bioactivity, half-life and potential immunogenicity are dependent on the degree of glycosylation, these results confirm the improvements obtained in the optimized more selective purification process, and the accuracy of optimized CZE method to monitor glycosylation pattern.

### 3.4.3. *Batch-to-batch reproducibility*

The optimized CZE method was used to assess lot-to-lot consistency in regard to glycosylation complexity. Two batches of purified rhIL-7 (B and C) were compared, batch C differs from batch B only by the strict application of cGMP manufacturing. The glycoform profiling obtained for batches B and C (Fig. 3B) are similar both qualitatively and quantitatively. For the two batches, percentages of the seven peaks were within the ranges defined previously (Table 6).

## 4. Concluding remarks

In this work, an accurate CZE method for profiling the intact rhIL-7 by CZE is presented. The validated method was performed under alkaline conditions combining sodium borate and the use of DAB as a dynamic coating agent. The careful optimization of the capillary conditioning afforded good repeatability, compatible with an application of the method to QC. In addition, the specificity of the developed CZE method was demonstrated. It is able to discriminate glycosylated rhIL-7 (from CHO) from matrix formulation and batches with poor glycosylation from those exhibiting highly complex glycosylation. The new CZE method allows quality assessment of the purified recombinant protein, quantification and profiling of glycoforms of rhIL-7, and thus selection of the appropriate batch of rhIL-7 during product release step as well as batch-to-batch consistency control.

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