



# Monoclonal antibody N-glycosylation profiling using capillary electrophoresis – Mass spectrometry: Assessment and method validation



Jérémie Giorgetti<sup>a</sup>, Valentina D'Atri<sup>b</sup>, Julie Canonge<sup>a</sup>, Antony Lechner<sup>a</sup>, Davy Guillarme<sup>b</sup>, Olivier Colas<sup>c</sup>, Elsa Wagner-Rousset<sup>c</sup>, Alain Beck<sup>c</sup>, Emmanuelle Leize-Wagner<sup>a</sup>, Yannis-Nicolas François<sup>a,\*</sup>

<sup>a</sup> Laboratoire de Spectrométrie de Masse des Interactions et des Systèmes (LSMIS), UMR 7140 (Unistra-CNRS), Université de Strasbourg, France

<sup>b</sup> School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Centre Médical Universitaire (CMU), Rue Michel-Servet 1, 1206 Geneva, Switzerland

<sup>c</sup> Centre d'immunologie Pierre Fabre, Saint-Julien-en-Genevois, France

## ARTICLE INFO

### Keywords:

Capillary electrophoresis-mass spectrometry  
Monoclonal antibody  
IgG glycosylation  
HILIC-FD  
Glycopeptide

## ABSTRACT

Characterization of therapeutic proteins represents a major challenge for analytical sciences due to their heterogeneity caused by post-translational modifications (PTM). Among these PTM, glycosylation which is possibly the most prominent, require comprehensive identification because of their major influence on protein structure and effector functions of monoclonal antibodies (mAbs). As a consequence, glycosylation profiling must be deeply characterized. For this application, several analytical methods such as separation-based or MS-based methods, were evaluated. However, no CE-ESI-MS approach has been assessed and validated. Here, we illustrate how the use of CE-ESI-MS method permits the comprehensive characterization of mAbs N-glycosylation at the glycopeptide level to perform relative quantitation of N-glycan species. Validation of the CE-ESI-MS method in terms of robustness and reproducibility was demonstrated through the relative quantitation of glycosylation profiles for ten different mAbs produced in different cell lines. Glycosylation patterns obtained for each mAbs were compared to Hydrophilic Interaction Chromatography of 2-aminobenzamide labelled glycans with fluorescence detector (HILIC-FD) analysis considered as a reference method. Very similar glycoprofiling were obtained with the CE-ESI-MS and HILIC-FD demonstrating the attractiveness of CE-ESI-MS method to characterize and quantify the glycosylation heterogeneity of a wide range of therapeutic mAbs with high accuracy and precision.

## 1. Introduction

Monoclonal antibodies (mAbs) were introduced for the treatment of various diseases in the late 1980 and they still represent the most rapidly growing category of therapeutic molecules today [1–3]. mAbs are particularly interesting because of their good therapeutic efficiency, favorable pharmacokinetic and pharmacodynamics, and relatively low side-effects [4]. mAbs are tetrameric glycoproteins having a molecular mass of approximately 150 kDa, composed of two heavy chains and two light chains, inter-linked by several disulfide bonds, and having at least one conserved N-glycosylation site located in the Fc domain [3]. Glycosylation is a post-transcriptional modification (PTM) that occurs naturally during excretion of antibodies from the expression system to the extracellular medium. It only represents 2–5% of the total mass of the protein but it is subjected to extensive studies due to its significant influence on effector functions of mAbs, especially antibody-dependent

cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) [5–8]. As a consequence, the mAbs glycosylation profile is considered as a critical quality attribute (CQA) and must be thoroughly analyzed [9–13]. The complexity and heterogeneity of the glycosylation pattern is mainly due to mAbs production in living expression systems [14–16] and requires a number of orthogonal analytical techniques to be fully characterized. Several analytical methods have been described for the glyco-variants characterization at different levels (from released glycans to intact protein level) including separative techniques (liquid chromatography (LC), capillary electrophoresis (CE)) often coupled to spectrometric, amperometric and mass spectrometric detection [17–21]. Recently, Reusch's group published two major articles dealing with the analysis of Fc-glycosylation profiles, and comparing several separation methods hyphenated or not with mass spectrometry (MS) detection [20,21]. This comprehensive comparison showed an excellent precision and accuracy for all the methods.

\* Corresponding author.

E-mail address: [yfrancois@unistra.fr](mailto:yfrancois@unistra.fr) (Y.-N. François).

However, concerning MS-based methods [22–25], a large panel of methodologies were evaluated, except the CE-ESI-MS approach. Nevertheless, in 2008, Gennaro et al. described the development of CE-ESI-MS technology with online LIF detection that allows identification of major and minor glycan species observed in the routine CE-LIF assay. Despite significant instrumental development to achieve LIF and MS dual detection, their strategies allowed to perform quantitative analysis provided by the on-line LIF trace and to increase confidence by providing accurate mass information [26]. More recently, Gahoual et al. reported the development of CE-ESI-MS technique for the characterization of the primary structure of mAbs performed in a single injection [27]. Based on a bottom-up approach, they highlighted the benefits of using electrophoretic separation in complement to chromatographic separation, which is conventionally applied in this type of study. CE separation selectivity allowed to simultaneously perform the amino acid sequencing and the PTMs characterization, including the N-glycosylation profiling. However, concerning the latter point, the approach was not statistically validated and potential bias in the obtained glycosylation heterogeneity could exist [28]. In the meantime, Heemskerck et al. reported the use of a similar methodology for highly sensitive IgG1 glycosylation profiling as a complementary method to a high-throughput nano-RPLC-MS [29]. They concluded that CE-ESI-MS provide information on IgG Fc glycosylation with concentrations below the LODs of the conventional methods. However, no comparison with reference method were applied to validate the obtained glycosylation heterogeneity.

In this report, we evaluated and validated CE-ESI-MS method to characterize and quantify the heterogeneity of the glycosylation pattern with high accuracy, precision and robustness. A systematic characterization study of glycopeptides obtained from ten different therapeutic mAbs produced in different expression systems (CHO, NS0 and SP2/O), has been performed to evaluate the suitability of CE-ESI-MS method, according to mAbs properties. Rituximab (chIgG1, CHO), palivizumab (hzIgG1, SP2/O), natalizumab (hzIgG4, NS0), nivolumab (huIgG4, CHO), trastuzumab (hzIgG1, CHO), panitumumab (huIgG2, CHO), adalimumab (huIgG1, CHO), infliximab-Remicade® (chIgG1, SP2/O) as well as two infliximab biosimilars, infliximab-Inflectra® (chIgG1 SP2/O) and infliximab-Reimsima® (chIgG1 SP2/O), were selected for this study. Comparison with glyco-profiling of released and 2-AB labelled glycans (used as a reference method) obtained by state-of-the-art Hydrophilic Interaction Chromatography (HILIC) was methodically performed to assess the reliability of the CE-ESI-MS methodology.

## 2. Experimental

### 2.1. Chemicals

Chemicals used were of analytical grade or high purity grade and purchased from Sigma-Aldrich (Saint Louis, MO, USA). Water used to prepare buffers and sample solutions was obtained using an ELGA purelab UHQ PS water purification system (Bucks, UK). RapiGest SF surfactant was purchased from Waters (Milford, MA, USA). mAbs and biosimilars products were all kindly provided by Pierre Fabre laboratories (Saint-Julien en Genevois, France).

### 2.2. Sample preparation

A volume corresponding to 100 µg (0.67 nmol) of protein was used. Samples were first diluted from stock solution to a concentration of 45.6 µM by using milliQ water. A second dilution to a concentration of 22.2 µM was performed by using 0.1% RapiGest surfactant and incubation at 40 °C for 10 min. Reduction of the samples was then achieved by the addition of dithiothreitol (DTT, final concentration of 25 mM) and incubation at 95 °C for 5 min. Once cooled down to room temperature (RT), the alkylation of the cysteines (Cys) was performed to avoid the reformation of the disulfide bonds. Thus, iodoacetamide

(IDA, final concentration of 10 mM) was added to the samples, followed by incubation at RT for 20 min in the dark. For performing the trypsin digestion, a volume of 1 µL of trypsin (0.5 µg/µL) was added to the samples that were left at room temperature for 3 h. Then another volume of 1 µL was added afterward and digestion was performed overnight at 37 °C. In order to cleave the surfactant, formic acid (FA) was added to the samples at a final concentration of 1% (v/v) and samples were left at RT for 2 h. Samples were finally diluted to a final protein concentration of 2.2 µM using ammonium acetate 50 mM (pH 4.0).

### 2.3. Capillary electrophoresis

The CE experiments were performed with a CESI8000 capillary electrophoresis system from Sciex Separation (Brea, CA, USA). A 32 Karat™ (Sciex Separation) was used for instrument control, data acquisition and data handling. Bare fused-silica capillaries (total length 100 cm; 30 µm i.d.) with characteristic 3 cm porous tip on its final end, a second capillary (total length 80 cm; 50 µm i.d.) filled during experiments with BGE allows electric contact. New capillaries were flushed for 10 min at 75 psi (around 5 bar) with methanol, then 10 min with 0.1 M sodium hydroxide, followed by 10 min with 0.1 M hydrochloric acid and water for 20 min at 75 psi. Finally, the capillary was flushed 10 min at 75 psi with BGE which contains 10% acetic acid. Hydrodynamic injection (6 psi for 2 min) corresponding to a total volume of 90 nL of injected sample was used. Injection volumes were calculated by using the CEToolbox application (Pansanel, GooglePlay). Separations were performed using a voltage of +20 kV.

### 2.4. Mass spectrometry

For glycopeptide analysis, the CESI system was hyphenated with a 5600 TripleTOF mass spectrometer (Sciex, Darmstadt, Germany). The MS instrument is equipped with a hybrid analyzer composed of quadrupoles followed by a time-of-flight (TOF) analyzer. ESI source parameters were set as follows: ESI voltage – 1.75 kV, gas supplies (GS1 and GS2) were deactivated, source heating temperature 150 °C and curtain gas value 5. Experiments were performed in Top15 information dependent acquisition (IDA), accumulation time was 250 msec for MS scans and 100 msec for MS/MS scans leading to a total duty cycle of 1.75 s. Mass/charge ( $m/z$ ) range was set to 100–2000 in MS and 50–2000 in MS/MS. Using those parameters, the mean resolution provided by the instrument is 40,000 in MS (for  $m/z$  485.251) and 25,000 in MS/MS (for  $m/z$  345.235).

### 2.5. MS/MS data analysis

Data obtained from the sheathless CE-MS/MS experiments were analyzed using Peakview software (Sciex, San Francisco, CA). The allowed mass tolerance for search algorithm identification, were set to  $\pm 5$  ppm and  $\pm 0.05$  Da for precursor ions and fragmentation ions, respectively.

### 2.6. HILIC (2-AB)

Each mAb (200 µg; 1.34 nmol) was deglycosylated by incubation with 2 µg PNGase F (500,000 U/mL, New England Biolabs) at 37 °C for 3 h following the standard protocols provided by the manufacturer. Released glycans were labelled with 2-AB at 65 °C for 3 h (Glyko Signal 2-AB Labeling Kit, ProZyme), and then purified using dedicated GlikoClean S Cartridges (ProZyme). Labelled glycans were washed with 96% acetonitrile (ACN), eluted from the cartridges and evaporated to dryness using a speedvac. Samples were reconstituted in 100 µL of 30:70 water/ACN (v/v). The pH of the mobile phase was 4.5. Analysis were performed by HILIC using an Agilent AdvanceBio Glycan column (2.1 × 150 mm, 1.8 µm) on a Waters ACQUITY UPLC I-Class system equipped with a binary solvent delivery pump, an auto-sampler, a UV-

DAD and a fluorescence detector (FD) set at  $\lambda_{\text{ex}} = 260$  nm and  $\lambda_{\text{em}} = 430$  nm. The system included a flow through needle (FTN) injection system with a 15  $\mu\text{L}$  needle. Data acquisition, data handling and instrument control were performed with Empower 2 (Waters, Milford, MA, USA). Mobile phase consisted of 20 mM Ammonium Formate solution (A) and ACN (B). The column temperature was set to 55  $^{\circ}\text{C}$ , and injection volume was 2  $\mu\text{L}$  corresponding to 0.08–0.2  $\mu\text{g}$  glycan sample. The flow rate was set to 0.5 mL/min, and the gradient conditions consisted of 80–60% B in 25 min, followed by a 3 min washing step at 20% B and a 15 min re-equilibration step. Peaks were manually integrated and relative glycan compositions were calculated. For the correct identification of the labelled glycans, UHPLC-MS analysis were also performed using an ACQUITY UPLC system (Waters), equipped with a binary pumping system and fixed loop injector of 5  $\mu\text{L}$ . This UHPLC was coupled to a fluorescence detector (FD) and an electrospray time-of-flight mass spectrometer (Xevo<sup>™</sup> Q-ToF, Waters). The mass spectrometer was operated in the positive ion mode and ions were scanned over an  $m/z$  range of 500–2500 with a 1 s scan rate. Capillary voltage was set to 3.0 kV, sample cone voltage to 35 V, source temperature to 120  $^{\circ}\text{C}$ , desolvation gas temperature to 350  $^{\circ}\text{C}$  and gas flow to 800 L/h. The instrument was calibrated using the singly charged ions produced by a 2  $\mu\text{g}/\mu\text{L}$  sodium iodide solution in 2-propanol/water (1:1). Data acquisition and analysis were performed with MassLynx 4.1 (Waters). In all cases, a 2-AB labelled dextran ladder standard (1 pmol/ $\mu\text{L}$ ) was also injected before and after a series of 5 samples, to check the repeatability of the injections and verify that no drift in retention times was observed.

### 3. Results and discussions

#### 3.1. Characterization of N-glycopeptide by CE-ESI-MS

mAbs are glycosylated proteins whose N-glycans are naturally incorporated in the protein during secretion into the extracellular environment. Extensive glycans characterization in terms of structure and relative abundance is of prime importance. The main goal of this study was to demonstrate the possibility to use CE-ESI-MS method for performing glycosylation profiling of a large panel of therapeutic mAbs using glycopeptides MS data. Therefore, we realized a systematic study of glycosylation characterization on ten different mAbs produced in CHO, NS0 and SP2/0 cell lines (Table 1). To evaluate the viability of CE-ESI-MS, we focused our characterization on the main Fc N-glycan species typically found in therapeutic IgG mAb produced in the above mentioned cell lines and listed in Table 1. In this section, natalizumab results have been selected as example to describe the CE-ESI-MS methodology allowing to assess the in-depth N-glycan characterization. Fig. 1a illustrates the separation obtained for the tryptic digest of natalizumab for a 200 fmol injection. The total analysis time was less than 45 min and the resulting electropherogram showed that all peptides migrated between 15 and 40 min. As tandem MS data interpretation and peptide identification was automatically done using Mascot, to obtain fast and accurate data treatment, it was necessary to manually evaluate the CE-ESI-MS/MS data in order to identify the glycopeptides and determine their structures. Peak assignment of glyco-structures was performed based on accurate mass measurement in MS1, provided by high resolution MS (mass accuracy below 2 ppm) (Fig. 1b) and collision induced decay (CID) fragmentation spectra (Fig. 1c), respectively. Indeed, MS/MS spectra exhibited the fragmentation of glycan moieties present on the glycopeptide, giving structural information on the glycans along with reinforcing the confidence of the identification. Furthermore, the obtained electropherogram showed the separation of several glycopeptides, demonstrating the benefit of using CE for such characterization. The charge-based CE separation allowed the baseline resolution of sialic acid and neutral glycans located on the peptide EEQFN<sup>300</sup>STYR. Fig. 1a represents two windows on the electropherogram, corresponding to the neutral glycans separation (between

30.5 and 31.9 min) and to the sialic acid moieties separation (between 34.9 and 36.5 min). Moreover, particular glycopeptides having a difference of only one galactose could also be baseline separated. Glycopeptides having such a small difference in mass tend to compete against each other during the ionization process, potentially interfering with relative quantification, thus the capacity to separate them is clearly an intriguing advantage. To perform the glycans profiling of each mAb, relative occurrence levels were estimated from the sum of isotopic peak intensities, considering all charge states of the ion corresponding to one glycopeptide (Fig. 2a and Table S1). A comparison of all glycoforms abundance was then realized (Fig. 2b). To validate the method reproducibility, the digestions of each mAb were performed three times using different experimenters and triplicate injections of each digested sample were carried out. The relative occurrence glycan levels reported in Table 1 were calculated for a selected mAb as the average of all relative abundances of the nine values (three digestions and three injections per digested samples), with a confidence interval of 95%.

#### 3.2. Evaluation of CE-ESI-MS method performance

To assess the performance of the CE-ESI-MS methodology with respect to accuracy and precision, each mAb glycosylation profile was compared with the reference method, namely HILIC-FD glycosylation profile obtained upon release and 2-AB-labeling of the glycans. HILIC-FD acquisitions were performed in triplicate and peaks on the FD chromatogram were manually integrated for estimating the relative glycan compositions. The deviations observed for retention times were minimal ( $\text{RSD}_{\text{RT}} = 0.39\%$ , calculated on G0F, for  $n = 30$ ). Peak assignment of the 2AB-glycans was accomplished by online coupling of HILIC with ESI-MS. A detailed list of the glycan composition and the theoretical masses of the unlabelled/labelled glycans is shown in Table 2. The theoretical 2AB-glycan masses were used to obtain the extracted ion chromatogram (EIC) of each glycan. Furthermore, GlycoMod software (<http://web.expasy.org/glycomod/>) was eventually used for the prediction of the possible glycan structures based on the experimentally determined masses.

Table 1 compiles the results of CE-ESI-MS and HILIC-FD (2-AB) relative abundance values obtained for each mAb. As described in the literature [20], HILIC-FD shows excellent precision with low standard deviations (with the exception of Infliximab-Remsima<sup>®</sup> analysis). The suggested CE-ESI-MS method also presents low absolute variation with values below 4% for the different glycan structures. These values are comparable to those determined for other MS-based methods, such as NanoLC-ESI-MS described elsewhere [21]. It is worth noticing that for each mAb, the deviations were obtained based on the combination of digestions and injections performed in triplicates by different experimenters over an extended period of several weeks, thus the results strongly support the performance of the method in terms of robustness and reproducibility. Moreover, the relative occurrence level estimated by CE-ESI-MS method were in good agreement for the values obtained with the reference HILIC-FD method. For G0F and G1F, which represent at least 75% of the total glycosylation of each mAb, the relative absolute difference between CE-ESI-MS means and HILIC-FD means expressed as a percentage ( $100 \times |\text{Mean}_{\text{CE}} - \text{Mean}_{\text{HILIC}}| / \text{Mean}_{\text{CE}}$ ) showed an average of 7.5% for G0F and 7.8% for G1F. These values confirm the good fit between the two methodologies for the major forms of glycosylation. Moreover, for glycosylation expressed at least at 10% of the total glycoforms (i.e. G2F of Palivizumab), this value is up to 20% meaning a good variability between CE-ESI-MS and HILIC (2-AB) mean amount. For glycosylation representing less than 10% of the total glycoforms, the relative absolute differences between HILIC-FD and CE-ESI-MS are no longer representative, because these values can be very high, due to low degree of expression.

The relative quantification of mono-antennary structures, defined by the lack of N-acetylglucosamine (G0F-N, G1F-N, G0-N, G1-N), is known to be problematic using MS-based methods. Indeed, in-source



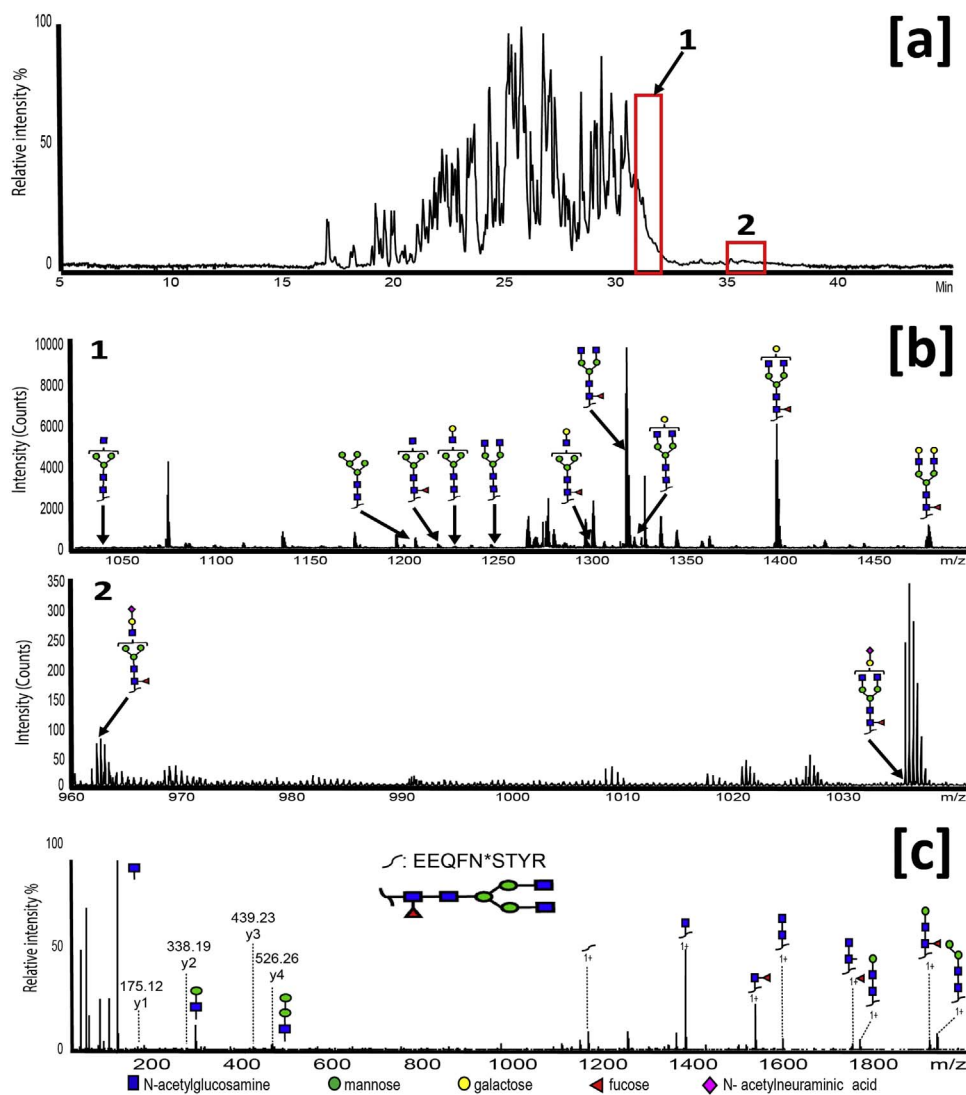


Fig. 1. (a) Base Peak Electropherogram corresponding to the analysis by CE-ESI-MS/MS of natalizumab tryptic digest. (b) MS spectrum of 30.5–31.9 min and 34.9–36.5. (c) MS/MS fragmentation spectra of [EEQFNSTYR] + G0F. Experimental conditions described in Experimental section.

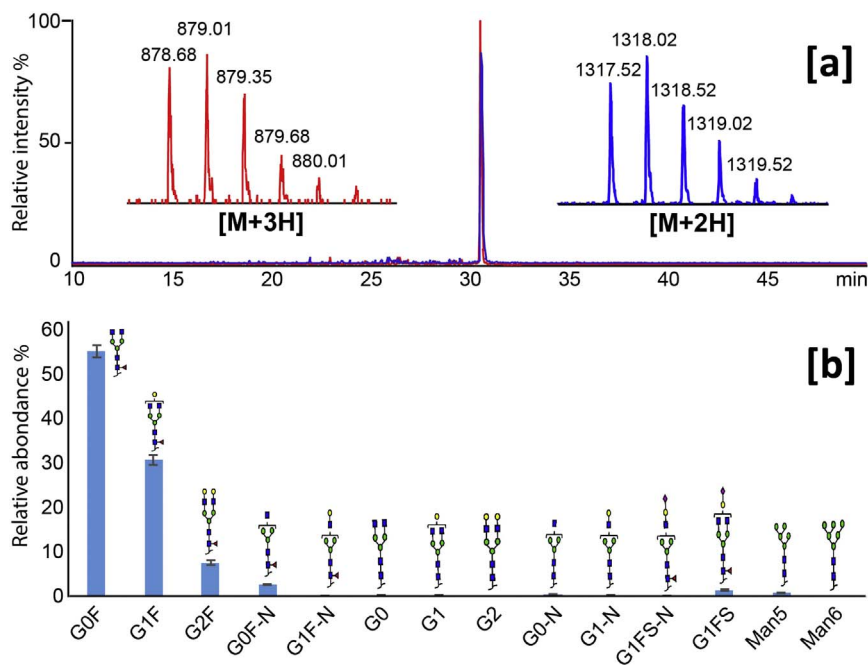


Fig. 2. (a) Extracted Ion Electropherogram (EIE) of  $m/z$  ratios 878.68 and 1317.52 ([EEQFN\*STYR] + G0F) and corresponding MS/MS fragmentation spectra (right-hand side). (b) Glycoforms relative abundance results obtained through the CE-ESI-MS data for the natalizumab Fc glycopeptide.

**Table 2**  
Overview of 2AB-glycans masses of monoclonal antibody.

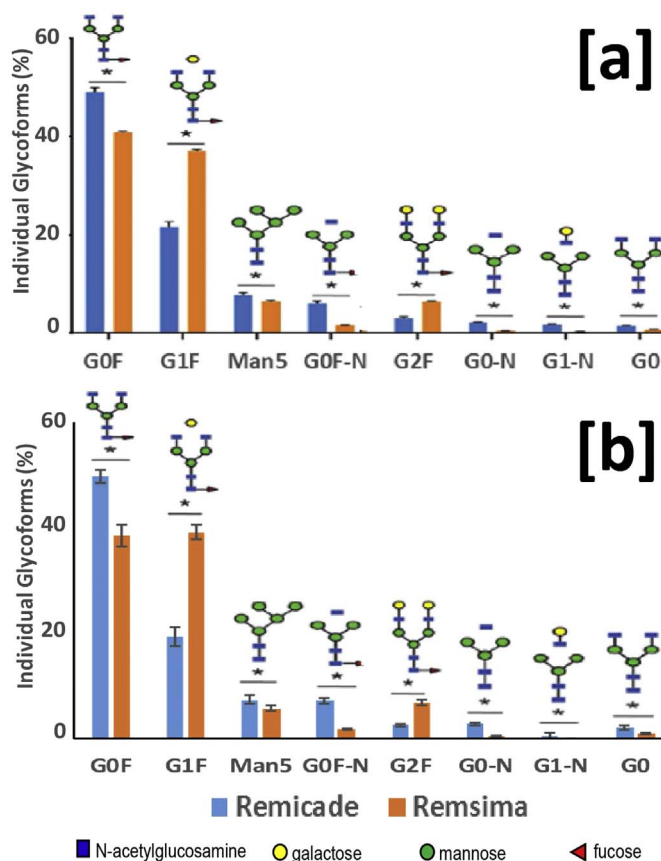
Glycan Species	Structural Scheme	Theo M	2-AB M	z=1 (+)	z=2 (+)	z=3 (+)
G0F [H3N4F1]		1444.534	1582.750	1583.757	792.382	528.590
G1F [H4N4F1]		1606.587	1744.803	1745.810	873.409	582.608
G2F [H5N4F1]		1768.640	1906.856	1907.863	954.435	636.626
G0F-N [H3N3F1]		1241.455	1379.671	1380.678	690.843	460.897
G1F-N [H4N3F1]		1403.507	1541.723	1542.730	771.869	514.915
G0 [H3N4]		1298.476	1436.692	1437.699	719.353	479.904
G1 [H4N4]		1460.529	1598.754	1599.752	800.380	533.922
G2 [H5N4]		1622.582	1760.798	1761.805	881.406	587.940
G0-N [H3N3]		1095.397	1233.613	1234.620	617.814	412.211
G1-N [H4N3]		1257.449	1395.665	1396.672	698.840	466.229
G1FS-N [H4N3FS1]		1710.598	1848.814	1849.821	925.414	617.178
G1FS [H4N4FS1]		1913.677	2051.893	2052.900	1026.954	684.971
M5 [H5N2]		1216.423	1354.639	1355.646	678.327	452.553
M6 [H6N2]		1378.476	1516.692	1517.699	759.353	506.571

fragmentation of bi-antennary structures resulting in the loss of one antenna can generate elevated mono-antennary structures levels, along with a charge reduction that is commonly observed in MS spectra and that is a consequence of a loss of the N-acetylglucosamine [21]. While only low or similar levels of mono-antennary structures were detected in CE-ESI-MS for eight mAbs, higher levels of these glycovariants were detected for natalizumab and nivolumab, as compared to HILIC-FD (Table S2). However, for these two mAbs, differences between the mono-antennary averages obtained by CE-ESI-MS and HILIC-FD were not aberrant and no charge state reduction was observed by CE-ESI-MS. This behaviours mean that the detected sum of mono-antennary structures were not over-estimated. Moreover, this result confirmed that during ESI-MS analysis of glycopeptides, in source decay can efficiently be avoided through the proper choice of the MS conditions and voltages, even for CE-ESI-MS method [21].

The sum of afucosylated species (G0, G1, G2) is a relevant parameter for antibody effector function. For nine mAbs, similar levels of G0 + G1 + G2 were observed. Only trastuzumab showed a difference between CE-ESI-MS (8.3%) and HILIC-FD (4.4%) (Table S2). Similarly, the sum of highly mannosylated species (M5, M6) was evaluated and similar levels of M5 + M6 were observed for seven mAbs, along with small differences concerning M5 species for adalimumab, infliximab-Remicade® and palivizumab. This variability, already observed with other MS-based methods [21], could be explained by the low degree of expression of N-glycans. Finally, good correlations were observed for the mean levels of sialylated structures sum (G1FS, G1FS-N). Overall comparisons of results obtained by CE-ESI-MS approach and HILIC-FD reference method showed very similar glycoprofiling of the ten therapeutic mAbs. CE-ESI-MS demonstrated to be a valuable method to characterize and quantify with high accuracy, precision and robustness the most largely expressed glycan species as well as the low abundance glycoforms.

Recently, Pisupati et al. published an important work describing a multidimensional analytical comparison of infliximab-Remicade® and the biosimilar infliximab-Remsima® [30]. They performed the glycoforms quantification by LC-MS using trypsin-digested products and demonstrated, for the first time, significant differences in the N-Glycan distributions for infliximab-Remicade® and infliximab-Remsima® (Fig. 3a). To confirm the assessment of CE-ESI-MS method in performing the relative quantitation of mAbs glycopeptides, we compared

our infliximab-Remicade® and infliximab-Remsima® means with those obtained by Pisupati et al. It must be highlighted that no collaboration was carried out between Prof. Schwendeman's group and our



**Fig. 3.** Comparison of Infliximab-Remicade® and infliximab-Remsima® glycoprofiling. (a) Quantification of N-glycans adapted following the method of Pisupati et al. (adapted with permission from [30]. Copyright (2017) American Chemical Society. (b) Quantification of N-glycans following CE-ESI-MS analysis.

laboratory, which means that samples were not from the same batch, and that experimenters and instrumentations were different. Fig. 3b represents the glycoprofiling of infliximab-Remicade® and infliximab-Remsima® following CE-ESI-MS analysis for the selected N-glycan species. Comparison between Pisupati et al. results and our profiles highlights a total similarity between the two glycoprofilings. For the eight selected N-glycan species, similar relative abundance have been obtained and originator/biosimilar comparison followed the same expression degree differences. While the confirmation of CE-ESI-MS performance in term of N-Glycan species quantification was proved, significant differences in the N-Glycan distributions for infliximab-Remicade® and infliximab-Remsima® were confirmed.

Infliximab-Inflectra® is another biosimilar that was studied in this work and not reported by Pisupati et al. Thanks to the CE-ESI-MS methodology, we showed for the first time significant differences in the N-Glycan distributions also for infliximab-Remicade® and infliximab-Inflectra® (Table 1).

#### 4. Conclusions

To summarize, we reported here the development of a CE-ESI-MS methodology to perform relative quantitation of N-glycan species for mAbs characterization at the glycopeptides level. Validation in terms of robustness and reproducibility of CE-ESI-MS method were demonstrated through the relative quantitation of glycosylation profiles for ten different mAbs produced in different cell lines. A systematic comparison of the glycosylation patterns obtained for each mAbs was compared with that obtained with the HILIC-FD reference method. Results obtained with the CE-ESI-MS approach and HILIC-FD showed very similar glycoprofiling, demonstrating the attractiveness of CE-ESI-MS method to characterize and quantify the glycosylation heterogeneity of a wide range of therapeutic mAbs, with high accuracy and precision. In addition, focusing on the glycopeptide may allow to distinguish glycans with regard to their glycosylation sites, which cannot be done when using methodologies involving glycans release. This property may be important, especially in case of more complex glycoproteins or for mAbs having several glycosylation sites. Moreover, it must be mentioned that our CE-ESI-MS methodology is not restricted to the sole purpose of glycopeptides characterization and quantitation, but it can also be employed for the overall characterization of mAbs, including primary structure assessment with complete sequence coverage as well as identification and quantification of a large number of PTMs, all performed within a unique single analysis [27]. In our opinion, this work proves that CE-ESI-MS could be a viable alternative to LC-ESI-MS for glycosylation profiling and should be considered as an innovative approaches in MS-based proteomics applied to mAbs characterization.

#### Acknowledgments

Emmanuelle Leize-Wagner would like to thank Sciex for the fruitful collaboration and the support. The authors would like to express their gratitude to Camille Banholzer, Jordan Kirmann and Charly Renard (LSMIS, Université de Strasbourg, France), P. Hammann, Lauriane Kuhn and J. Chicher (Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France) and MC. Janin-Bussat (Centre d'Immunologie Pierre Fabre, St Julien en Genevois, France). This work was supported by the CNRS (UMR 7140), the University of Strasbourg. Davy Guillaume also wishes to thank the Swiss National Science Foundation for its financial support (31003A\_159494).

#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.talanta.2017.09.083>.

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