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Characterization and quantification of therapeutic monoclonal antibodies, anti-drug antibodies and their interactions for clinical applications

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A B S T R A C T

Therapeutic monoclonal antibodies (mAbs) are experiencing tremendous developments in terms of novel approved products and applications fields. Nonetheless, after administration, mAbs are submitted to a significant number of biological phenomena that may affect their quantity, structure and potency. Moreover, as mAbs are not exempt from adverse effects they can trigger immune responses leading to the expression of anti-drug antibodies (ADAs), potentially impacting their efficacy. This critical review details the different analytical techniques used for the characterization and quantification of mAbs and ADAs in different contexts from routine therapeutic drug monitoring to advanced clinical investigations. In addition, state-of-art approaches providing information regarding mAbs/antigen or mAbs/ADAs interaction such as binding affinities are described through impactful applications. Different analytical approaches are discussed including binding assays like ELISA and chemiluminescent immunoassay. Similarly, molecular interaction analyses are detailed like surface plasmon resonance and mass photometry. Finally, cutting-edge mass spectrometry-based analytical methods are presented with prior liquid chromatography and capillary electrophoresis separation, including the sample purification strategies required for the analysis of biological samples. Thus, the critical review discusses the technical requirements, advantages and limitations of the different analytical techniques in order to position them depending on the context and information required.

1. Introduction

Monoclonal antibodies (mAbs) have demonstrated an undeniable success as therapeutic agents. In 2024, over 125 different mAbs are approved for therapeutic use, and more than 200 products are currently in clinical trials [1]. Thus, their constant development over the last few years has modified the paradigm in the pharmaceutical industry, enabling the emergence of the biopharmaceutical field as an important actor. The relevance of therapeutic mAbs can be explained by their high specificity for their corresponding antigen. Also, their pharmacological properties, like extended serum half-life, are particularly interesting for therapeutic applications [2]. The expansion of therapeutic mAbs was initially driven by oncology and the treatment of immune diseases [3]. However, their applications are constantly broadening with the treatment of more common conditions like asthma [4]. In addition, the introduction of other formats derived from mAbs such as bispecific antibodies, antibody-drug conjugates and fusion proteins, are demonstrating the interest of this type of therapeutic protein [5].

Due to their recent introduction as therapeutic agents and the increasing number of mAbs, insights regarding their evolution after administration remain relatively limited. Thus, after injection of an equivalent dose, some patients show a rapid elimination of the mAbs whereas in a minor fraction of the treated population, they may be detected in the serum several month after administration [6]. In addition, mAbs are not exempt from side effects which may take various forms. In particular, over time, a majority of treated patients are developing natural antibodies targeting the mAbs product, referred as anti-drug antibodies (ADA) [7]. As a consequence, mAb based therapies require monitoring during treatment in order to provide an improved understanding regarding of mAbs long-term effects and efficacy.

A significant number of analytical methods are focusing on the analysis of purified mAbs production, however, they do not allow the characterization of mAbs after administration [8]. The growing interest regarding the study of the evolution of therapeutic mAbs after their administration currently drives the development of innovative analytical methodologies able to tackle the complexity of the biological samples analysis containing mAbs.

This review aims to provide a critical overview of the different analytical techniques used for the quantification and characterization of

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Abbreviations list

ABT	Antigen Binding Test
ADA	Antibody drug-antibody
AE	Acridinium Ester
ALP	Alkaline Phosphatase
CDR	Complementary-Determining Region
CE	Capillary Electrophoresis
CID	Collision-Induced Dissociation
cIEF	Capillary Isoelectric Focusing
CL	Chemiluminescence
CLIA	Chemiluminescent Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
ESI	Electrospray
ETD	Electron-Transfer Dissociation
F_{ab}	Fragment antigen-binding
FC	Flow Cytometry
F_c	Fragment crystallisable
HRP	Horseradish Peroxidase
IFX	Infliximab
IP	Immunoaffinity purification
Ig	Immunoglobulins
K_D	Affinity Constant
k_{off},k_d	Dissociation Constant
k_{on},k_a	Association Constant
LBA	Ligand Binding Assay
LC	Liquid chromatography
LLOQ	Lowest Limit of Quantification
LOQ	Limit of Quantification
LOD	Lowest Limit of Detection
mAb	Monoclonal Antibody
mELISA	Microfluidic biosensor-based ELISA
MP	Mass Photometry
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NADA	Neutralizing Antibody Drug-Antibody
nNADA	Non-neutralizing Antibody Drug-Antibody
PTM	Post-Translational Modification
RIA	Radioimmunoassay
SPR	Surface Plasmon Resonance
SRM	Selected Reaction Monitoring
SIL	Stable-Isotope Labeled
TDM	Therapy Drug Monitoring

therapeutic mAbs, their corresponding ADAs and measure their affinities in biological matrices. The first part is focusing on binding assays which represent the earlier approach for the analysis of mAbs in biological samples. In a second part, recent developments regarding molecular interaction analyses are described, especially concerning the possibility of using this type of techniques to obtain quantitative information and measure mAbs/ADAs interactions. Finally, the latest advances concerning mass spectrometry (MS) based analysis for the quantification and characterization of mAbs and ADAs are discussed. Thus, a particular emphasis on the potential and future developments expected concerning MS analysis is discussed for this type of clinical applications.

1.1. Monoclonal antibodies

Therapeutic mAbs are based on immunoglobulin G (IgG), which as such represent large biomolecules (molecular weight ~150 kDa). Because of their extended serum half-life, IgGs are the immunoglobulins type of choice for mAbs production [9]. Thus, mAbs are heterodimeric molecules composed of four polypeptides: two identical light chains (~25 kDa each) and two identical heavy chains (~50 kDa each) as emphasized in Fig. 1. Each F_{ab} domain contains 3 complementary-determining regions (CDR) identified by a high degree of amino acid sequence variability and ensure mAb specificity for its antigen [10]. C_{H2} region incorporates a N-glycosylation site expressing a significant number of glycans whose structures can influence the effector functions of the antibody [11]. The different glycosylation forms of mAbs can affect their stability, immunogenicity and clearance rate [12,13]. The F_c part interacts with F_c receptors found at the surface of cells such as immune cells involved in immune responses. For instance, it interacts with the neonatal F_c receptor which is responsible for the extended half-life of the mAb [14].

MAbs are complex macromolecules susceptible to physical and chemical degradation such as aggregation, denaturation, or post-translational modifications (PTMs) [15,16]. Regarding PTMs, Asn deamidation, Asp isomerization, Met oxidation and C-terminal Lys clipping are some of the main types of modification. Their occurrence and modification level can have an impact on the stability and the potency of the mAb [17]. Also, the localization of PTMs is important as for example a change in the CDR region can have an influence on antigen binding affinity [18]. Therefore, PTMs including glycosylation represents critical quality attributes that are strongly regulated during the manufacturing and storage processes [19–21].

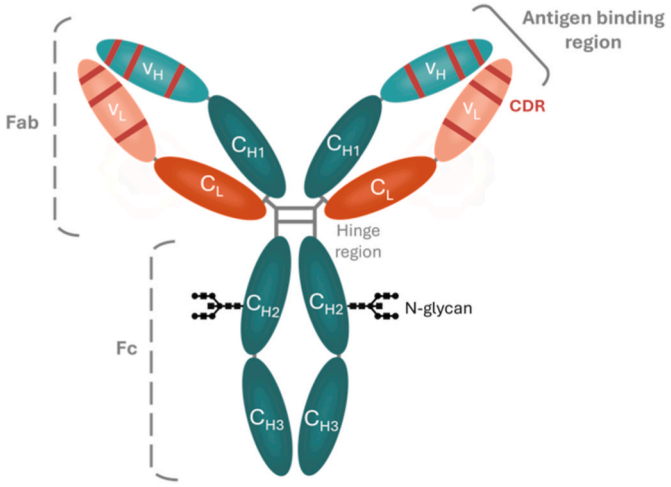

Fig. 1. Structure of an immunoglobulin G (IgG). The different types of IgG differ in disulfide bond numbers and positions.

Therapeutic drug monitoring (TDM) defines the clinical follow-up of treated patients after administration of the drug. Currently, TDM relies essentially on the quantification of free mAbs circulating in the patient's serum, potentially to adjust the administration periodicity and dosage [22]. However, there is a crucial need to study chemical and structural changes of mAbs occurring after administration. Thus, it could provide information from a different perspective like the occurrence of major PTMs or compare the clearance of the different glycoforms.

1.2. Anti-drug antibodies

The toxicity of mAbs is fairly limited, nevertheless, their administration may still trigger undesired side effects such as infection susceptibility, autoimmune diseases development and immunogenicity [23].

Immunogenicity defines the immune response after administration of a foreign substance, identified by the immune system as an exogenous agent. In the case of mAbs, the immune system may express anti-drug antibodies (ADAs), sometimes early in the course of the treatment [24]. With regard to the first generation of mAbs, major adjustments have been implemented to their structure, notably by limiting the incorporation of parts from non-human species in order to reduce their immunogenicity [25]. ADAs can be divided into two categories: non-neutralizing and neutralizing anti-drug antibodies (NADAs) as shown in Fig. 2. NADAs bind to the F_{ab} domain of the mAb which inhibits its interaction with the antigen [25]. Non-neutralizing ADAs (nNADA) bind to the F_c part of the mAb. This interaction induces the formation of immune-complexes with the mAb, which are rapidly cleared out of the system [26].

ADAs expression could be linked to abnormal decrease of the concentration of the mAb, a loss of response to the treatment or lower remission rates [28,29]. Also, the presence of residual ADAs after treatment can generate a negative response in case of further administration of another mAb, by increasing the sensibility to immune response [30,31]. Some mAbs have been identified as more prone to induce adverse immune response such as adalimumab and brolucizumab [27, 32].

Concerning TDM, essentially the presence and/or concentrations of ADAs are monitored in order to provide individualized treatments for each patient [33]. In the vast majority of mAbs treatment, ADAs production have been identified, however the impact of ADAs on the outcome of all treatments, for example in the case of cancer immunotherapy or new mAbs is still unclear [32,34]. In the case of infliximab, TDM in complement with adjusted dosing, has demonstrated to be more cost-effective than to increase the dose of the treatment in case of reduced response to the treatment [35]. However, ADAs analysis is particularly challenging because of their isotypes diversity and amino acid sequence variability. Nevertheless, detailed characterization of mAbs/ADAs complexes could be included to improve understanding of the interactions at the core of immune response in terms stoichiometry, affinity and localization.

2. Binding assay analysis

2.1. Enzymatic-linked immunosorbent assay

Enzymatic-linked immunosorbent assay (ELISA) represents the standard technique for the quantification of proteins in biological samples due to its swiftness, sensitivity, throughput and requires relatively simple equipment. ELISA was developed for the quantification of infliximab demonstrating great precision and accuracy for concentration ranging from 0.10 to 8 μg mL^{-1} corresponding to its therapeutic window [36]. Because of ELISA ease of use, relevant sensitivity and throughput for routine use, different kits were made commercially available for the quantification of the most common therapeutic mAbs, which include for example infliximab, adalimumab, rituximab, trastuzumab or ustekinumab [37]. However, commercial ELISA kits available represent only a small fraction compared to the extensive number of therapeutic mAbs approved.

ELISA can also be used for the quantification of ADAs, with bridging ELISA as the most common alternative [38,39]. The method was used for the detection of ADAs of infliximab, with the possibility to assign an immune response to a mAb concentration below 0.1 μg mL^{-1} [36]. ELISA experiments often need to be developed in house for the detection of ADAs. Bridging ELISA experimental design allows to address analytical biases originating from the expression of various ADAs isotypes depending on treated individuals or species of origin [40]. Although, it is difficult to correctly quantify IgG4 isotypes as they are considered monovalent due to their F_{ab} arm exchange process [41,42]. Another challenge may be the presence of the mAbs drug in the sample as it can cause an underestimate of the ADA due to drug-ADA complex formation. The drug interferences can be minimized by acidic dissociation. For example, Du *et al* developed a two-steps acid dissociation/bridging ELISA method for the detection of ADAs directed against anti-LAG3 mAbs [43]. Also, acidic dissociation could be used in order to achieve the ELISA detection of ADAs targeting cabiralizumab in the presence of 200 μg mL^{-1} of the mAbs [44]. Those experiments showed the possibility to identify the presence of ADAs even for samples containing high concentration of the drug with improved sensitivity and robustness [45]. Recent instrumental developments concerning ELISA for mAbs and ADAs quantification have focused on improving analytical performances, especially for use as point-of-care analysis. Iria *et al* have developed a microfluidic biosensor-based on ELISA (mELISA) designed to quantify infliximab in the plasma of treated patients (Fig. 3A). The mELISA required only 24 min to obtain the accurate and robust quantification of infliximab (Fig. 3BC), representing a drastic reduction of analysis time compared to conventional ELISA [46].

ELISA rapidly took the role of TDM reference technique for the quantification of mAbs and ADAs in biological samples, particularly due to the absence of sample preparation, the specificity of the incorporated ligand and the sensitivity of fluorescence detection. However, in some cases, its specificity may be compromised due to unspecific interactions between the ligand and the sample content, involving quantification biases to false positive in rare instances. Also, ELISA results showed significant variability indicating a need for standardization. Truffot *et al* compared two commercially available ELISA kits and liquid chromatography coupled to mass spectrometry (LC-MS) analysis for the

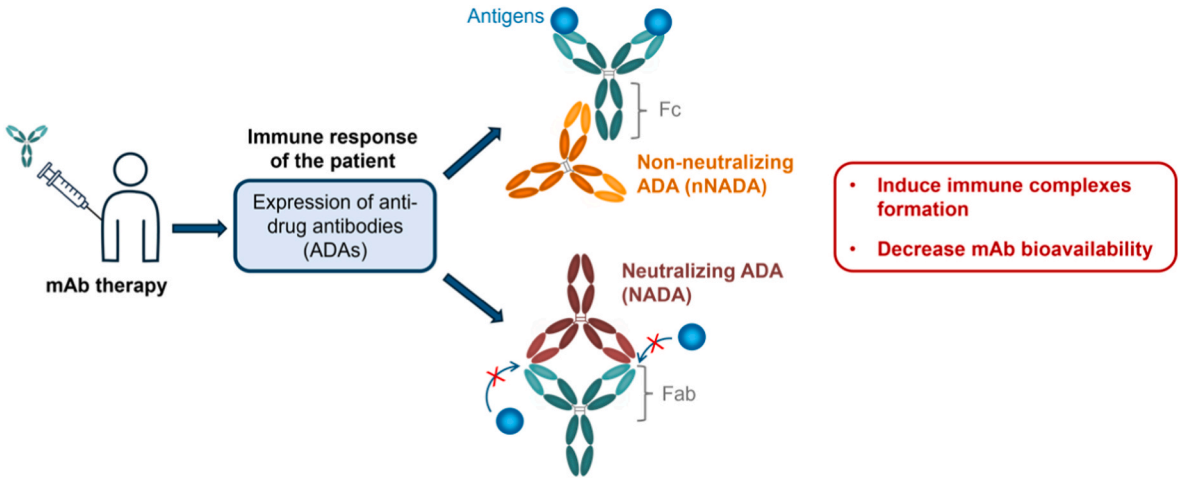

Fig. 2. Schematic representation of the two types of ADA synthesized by the immune response from immunogenicity.

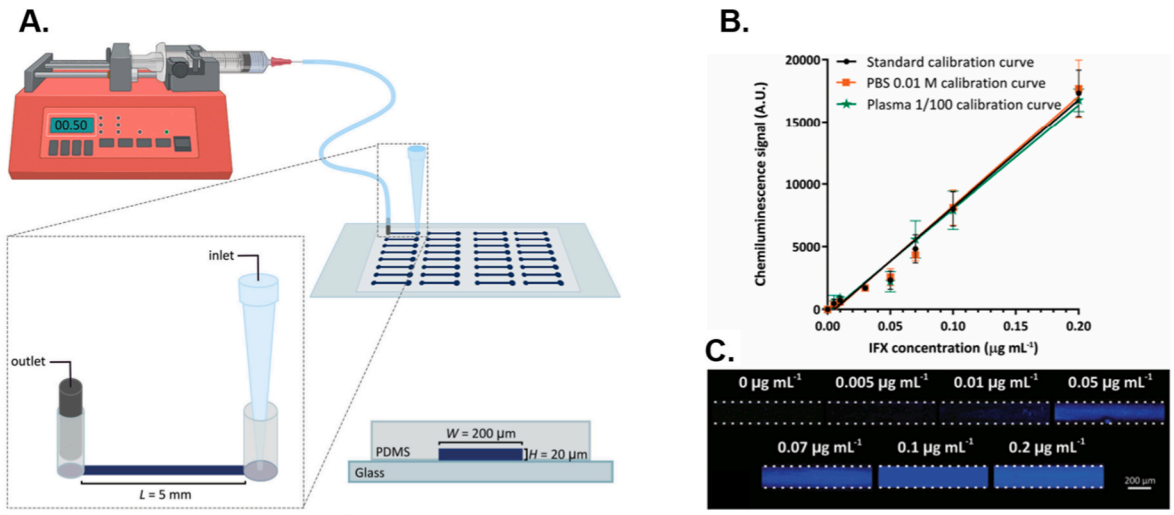

Fig. 3. Schematic representation of A) instrumental setup used for the mELISA, where cross-section diagram of the microchannel are illustrated, B) standard calibration curves of IFX in mELISA and C) representative signal intensity for IFX calibration curves in phosphate buffered saline (PBS) in mELISA. Adapted from Ref. [46].

quantification of rituximab and eculizumab. Results showed a negligible bias for ELISA quantification of rituximab, however the method demonstrated a significant bias of 69 % in the case of eculizumab [47]. Similarly, several publications described a systematic underestimation of mAbs concentration using ELISA [48].

Due to the specific nature of the ligand implemented for the capture of the mAb, ELISA experimental settings cannot be directly transposed to the quantification of several types of mAbs. Complete method development is necessary when developing a new ELISA assay. As a consequence, currently ELISA cannot provide the quantification of mAbs and the corresponding ADAs using a single experiment. Instead, samples need to be submitted to two different ELISA in order to obtain information regarding the mAbs and the ADAs respectively. Finally, ELISA cannot provide any information regarding the structure of the mAbs and/or ADAs. This characteristic may be particularly limiting especially to investigate the evolution of mAbs post-administration.

2.2. *Radioimmunoassay*

Radioimmunoassay (RIA) is a binding assay with the characteristic of using labeled biomolecules with gamma-ray emitting isotopes for the detection of the analyte [49]. For instance, the determination of ADAs in a biological sample can be done by adding radiolabeled $F(ab)_2$ fragments of the mAb they are targeting. The non-bound $F(ab)_2$ fragments are then washed and the radioactive signal of the complexes formed is measured as shown in Fig. 4 [50]. In other cases, the radiolabeled molecule that did not bound can also be measured and give a quantitative information on the complexes formed [51]. During the development of RIA assays, some characteristics of the radioligand (stability, lifetime) are important to study due to their influence on the experiment. Barta *et al* did an *in vitro* study on concentration, labelling efficiency and stability for ^{131}I-cetuximab using time-resolved RIA. They demonstrated the fast and efficient radioiodination of cetuximab using an incubation of 1 min, and the labelling showed to be stable up to 96h providing sufficient time to perform experiments [52]. They encourage the characterization of stability and the binding affinities of the labeled mAbs with real-time method before further use in RIA. ^{125}I and ^{131}I isotopes are relevant as radiolabeling agents because they combine high natural isotopic abundances and provide relevant sensitivity [53].

RIA could be successfully used for the quantification of edrecolomab

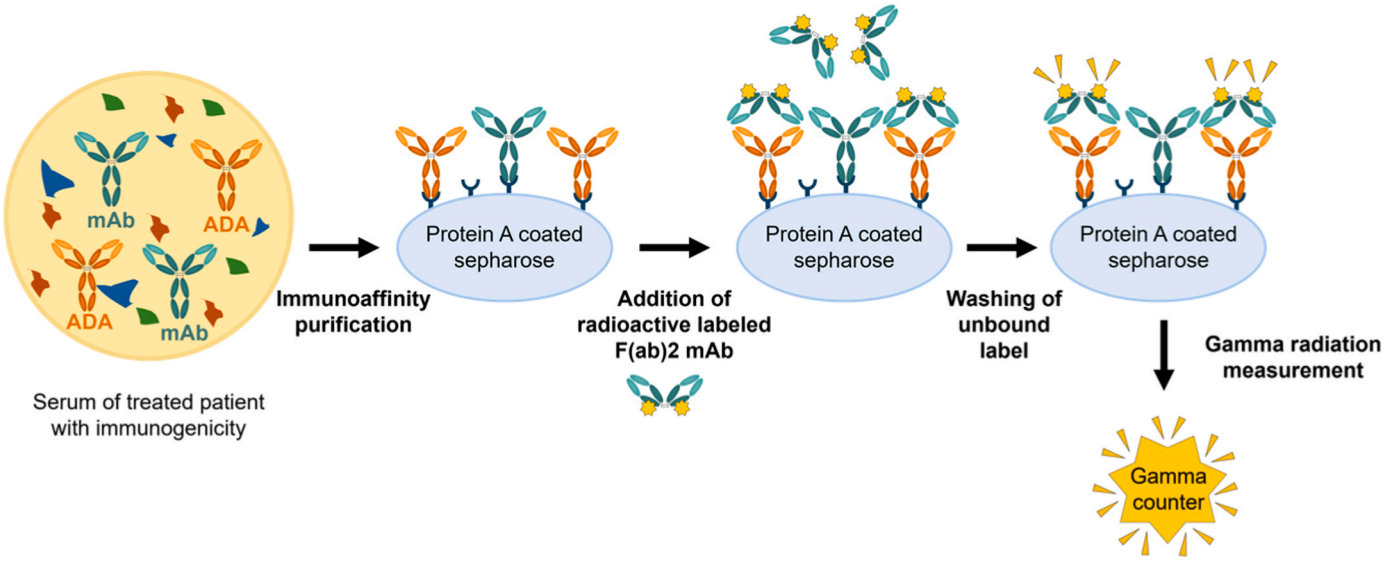

Fig. 4. Schematic representation of the radioimmunoassay strategy commonly used for the quantification of ADA in human serum.

from serum samples originating from patients treated for pancreatic cancer [54]. More recently, the implementation of RIA was described for the quantification of infliximab in serum [51]. Also, RIA could be successfully used to detect ADAs corresponding to adalimumab for rheumatoid arthritis samples from patients. Note, that the measurement of ADA and adalimumab levels proved to be an early predictor of the therapeutic response to the treatment [50].

Due to its experimental design, RIA allowed to reduce quantification biases compared to ELISA by limiting the impact of residual non-specific interaction. In addition, radioactive detection enables to achieve lower LOD whereas maintaining excellent specificity which is relevant for the analysis of mAbs and ADAs in biological samples [38]. Thus, for the detection of ADA, RIA sensitivity was also due an improved drug tolerance of the assay, associated with the co-presence of adalimumab [55]. However, safe handling of radioactive materials and the need for expensive equipment [56] represents important constraints that complexify its implementation compared to ELISA [52,55]. Still, the development of RIA allowed from a conceptual point of view, to pave the way in order to further improve the specificity and sensitivity of binding assays for the detection and quantification of mAbs and ADA.

2.3. Chemiluminescent immunoassay

Chemiluminescence (CL) is based on the detection of photons produced from a chemical reaction. This effect is used in chemiluminescent enzyme immunoassay (CLIA), where the species used for detection can generate light emission. For the analysis of mAbs and ADA, CLIA often relies on an antibody labeled with an enzyme. Typically, horseradish peroxidase (HRP) or alkaline phosphatase (ALP) is used with luminescent substrate like luminol for HRP or 1,2-dioxo-cyclohexane derivatives for APL [57]. The CL generated from HRP or APL reaction are usually not intense, thus various developments were realised to intensify the signal [58,59]. Darwish *et al* have developed an ultrasensitive CLIA for the quantification of durvalumab using HRP-luminol and hydrogen peroxide reaction in presence of 4-(1,2,4-triazol-1-yl)phenol [60]. Using a similar approach, they used 4-(1-imidazolyl)phenol as a CL enhancer for the CLIA quantification of atezolizumab achieving high sensitivity and compatible with high throughput analysis [61].

Enzymes used to perform CLIA can also create variabilities in term of reproducibility, significant background signal and potential inhibition of the interaction between antigen and antibody [62]. Another type of chemiluminescent reaction for mAbs quantification uses acridinium ester (AE) labeled antibodies, to remove the use of enzymes [63]. These characteristics have led to the commercialization of AE labeling kits, that were used for the analysis of infliximab, adalimumab and the corresponding ADAs [64]. Consequently, the AE-label CL method could be further integrated in an easy automated system for high-throughput analysis [65]. Compared to conventional ELISA and RIA methods, CLIA has recently attracted much interest due to its better sensitivity, wider dynamic range and low background [61,63]. In clinical practice, CLIA has gained attention due to its rapid analysis and the increase of standardization practice enabled by the automated process. Another major advantage has also been the possibility to analyse in biological samples multiple drugs or a mAb and its ADA in a single analysis [65].

3. Molecular interaction analysis

3.1. Flow cytometry

Flow cytometry (FC) is a real-time analysis technique based on the measurement of light scattering events and fluorescence emission of cells or particles in solution passing in front of a focused laser. FC provides quantitative and qualitative analysis of mAbs. FC quantification of mAbs could be achieved for rituximab, providing accurate and sensitive quantification for the concentration range 5–250 μg mL^{-1} [66]. Furthermore, FC could also be used to determine the affinity between adalimumab and its corresponding antigen TNF-α [67]. Affinity measurements using FC could be applied to compare different mAbs biosimilars, emphasizing potential differences in potency and functional differences [68]. Similarly, FC can be used to characterize the interaction of mAbs with effector function receptors, which can contribute to the optimization of mAbs design [69]. Also, recent advances further improved the ability of FC to evaluate nonspecific binding interactions between mAbs and non-antigen biomolecules in order to assess mAb polyspecificity [70].

FC could also be used to investigate mAbs immunogenicity in the form of ADAs expression. Thus, a FC method was developed for the simultaneous quantification of adalimumab and the corresponding ADAs in plasma samples. Using polystyrene beads functionalized with either TNF-α or adalimumab $F(ab')_2$, robust quantification could be demonstrated between 79-600 and 1–150 ng mL^{-1} for adalimumab and ADAs respectively [71]. Thereby, FC could potentially provide improved sensitivity for the quantification of mAbs or ADAs compared to conventional bridging ELISA, with reduced interferences from the matrix. Nevertheless, FC applications remain limited due to the complexity of the method development, which in addition requires detailed assessment in order to ensure absence of biases and cannot be transposed to different mAbs and/or ADAs.

3.2. Surface plasmon resonance

Surface Plasmon Resonance (SPR) is an analytical technique used to monitor molecular interactions in real time [72]. Thus, the monitoring of binding provided by SPR enables the experimental measurement of kinetic constants, especially the association rate (k_{on}, k_a) and the dissociation rate (k_{off}, k_d) corresponding to the interaction. Also, SPR sensorgrams allow to determine equilibrium dissociation constants (K_D) that define affinity. As a consequence, SPR has gradually become an essential tool for the characterization of protein-protein interactions.

SPR could be successfully used for the characterization of a mAb-antigen interaction. Wang *et al* developed a SPR method to determine the kinetic and affinity characteristics for the interaction between CD20 receptor and respectively rituximab or obinutuzumab F_{ab}. Thus, they demonstrated different CD-20 affinities of the two proteins with K_D measurements of 0.2 and 62 nM respectively [73]. Similarly, SPR could be implemented to measure the affinity between pembrolizumab and the targeted PD-1 receptor [74]. SPR provides crucial information regarding the affinity for the targeted antigen which showed to be suitable for the biosimilarity assessment in the case of biosimilar mAbs having distinct glycosylations profiles [75].

SPR could also be implemented for the detection and analysis of mAbs-ADAs interactions. For example, Real-Fernàndez *et al* developed a strategy to determine the affinity of anti-adalimumab antibodies present in the sera of treated patients using immobilized adalimumab [76]. Also, SPR could be used to measure the affinity of ADAs expressed in rats exposed to rituximab. The study also highlighted that ADAs expressed from different individuals exhibited variable affinities illustrating differences of immune response [77]. Similarly to binding assays, the presence of important quantities of mAbs in the sample may generate drug interferences when performing SPR analysis. Weeraratne *et al* showed the benefits of using a secondary confirmatory detector antibody to improve drug tolerance and signal sensitivity of the method. Results obtained for the fusion protein trebananib demonstrated a more accurate assessment of ADA pharmacokinetic with trebanabib than CLIA [78].

Various developments were recently reported to enrich the applicability of SPR. Beeg *et al* developed a novel SPR instrumental technology to enable the simultaneous quantification of a mAb and ADAs in a single analysis. On a single sensor surface, several flow channels were used to immobilize different types of ligands on parallel stripes, and samples were introduced in parallel over all the immobilized ligands (Fig. 5A). Therefore, multiple ligands and solutions could be measured in a single

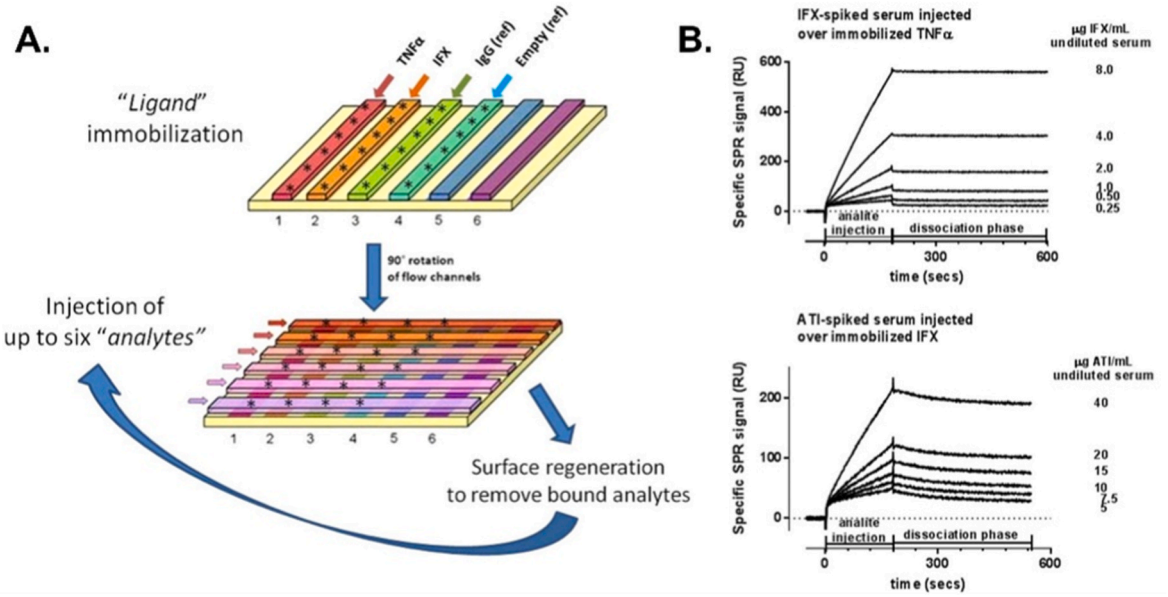

Fig. 5. A) General scheme of the SPR-based assay for simultaneous determination of infliximab (IFX) and antibodies directed against IFX (ATI) concentrations in serum. The SPR apparatus has six flow channels which can immobilize up to six ligands on parallel strips of the same sensor surface. B) Representative sensorgrams obtained injecting simultaneously six solutions of IFX-spiked sera over immobilized TNFα, or six solutions of ATI-spiked sera over immobilized IFX. Adapted from Ref. [79].

analysis (Fig. 5B) [79]. Consequently, they identified significantly higher concentration of ADAs and less false negative when performing quantification using SPR compared to commercial ELISA assay. These differences were attributed to long incubation times during ELISA experiment and the presence of low affinity ADAs in sample originating from patients as opposed to high affinity IgGs used for ELISA calibration [80]. To prevent similar phenomenon in SPR, a calibration-free SPR method was also developed for the quantification of ADAs in serum samples [81].

SPR has revealed to be a particularly powerful technique for the characterization of protein-protein interaction, capable to provide detailed characterization of such subtle phenomenon. Also, the instrumental versatility of SPR progressed significantly with the possibility to immobilize different types of partners. Such characteristics are interesting for mAbs and ADAs that interact with multiple antigens and receptors. SPR analysis is not impacted by eventual matrix effects and the method does not involve sample labeling or pretreatment. Moreover, due to the low sample preparation required and real time monitoring of interactions, this method has proven to be capable of detecting ADAs with lower affinities compared to ELISA for serum samples originating from patients treated using panitumumab [82]. The possibility of using SPR to measure precisely the affinity between a mAb and the corresponding ADA could be successfully correlated to the therapy outcome of patients [83]. As a consequence, SPR can provide information critically important for TDM, enabling an earlier discovery of ADA in order to provide more patient-specific therapeutic decisions.

3.3. Mass photometry

Mass photometry (MP) is an analytical technique recently introduced, capable to measure the mass of single biomolecules and complexes in solution. MP is based on an optical microscope system modified to measure the interference between light scattered by the solubilized analyte and light reflected from the surface. The analyte binds non-specifically to the cover glass surface of the microscope, resulting in a change in the refractive index at the interface between glass and water (Fig. 6A). The visual signal undergoes image processing (Fig. 6B) to obtain a measure of the interferometric contrast (Fig. 6C), which can be correlated to the molecular weight of the analytes (Fig. 6D). MP enables to estimate with a 2 % accuracy the molecular mass of compounds presents at nanomolar concentrations using only 10 µL of sample. Also, it does not require sample preparation and therefore represents a high-throughput technique as analysis time is commonly about 5–30 s per sample [84]. MP could be used to analyse a variety of biomolecules such as viral vectors [85], nucleic acid [86] and membrane proteins [87].

MP characteristics are particularly interesting for the study of antibodies because it allows direct observation of antibody binding events. Thus, MP could be successfully used to characterize the formation of complexes associating an anti-thrombin mAb to its target antigen or FCGR3A receptor respectively. Data allowed to determine unambiguously their different stoichiometries. Moreover, K_D dissociation constants could be calculated from measurement of free and bound analytes [88]. Another study highlighted differences of the k_{off} and k_{on} kinetic constants for the formation of complexes between glycosylated and deglycosylated trastuzumab with the FcγRIa receptor [89]. When compared to size exclusion chromatography-native MS and charge detection-MS for the analysis of protein-protein interactions, MP demonstrated the ability to overcome some limitations of native MS regarding the measurements of highly heterogeneous antibody-antigen co-occurring complexes [90]. Thus, MP could be implemented concomitantly with charge detection-MS for the characterization of the complex formed between SARS-CoV-2 proteins and IgGs. The study gave further information on the binding behaviour of IgGs towards the SARS-CoV-2 and the kinetics of the different stoichiometry complexes that are formed [91].

MP was also used to characterize the interaction between mAbs and ADAs. Reinert *et al* used MP to characterize the interaction between infliximab and a corresponding ADA. MP data allowed to precisely determine the stoichiometries of the resulting complexes and demonstrated the specificity of infliximab-ADAs interaction (Fig. 7A). Finally, study of the infliximab-ADAs complexes formation according to the molar equivalent of the two species could be achieved (Fig. 7B) [92]. Regardless of its recent introduction, MP represents a quite promising technique for the characterization of mAbs and ADAs.

The study of biomolecular interactions between a mAb and its target is important, for example to determine their stoichiometries and their affinities. By following the formation of complexes in solution, MP provides highly accurate information on the binding affinity,

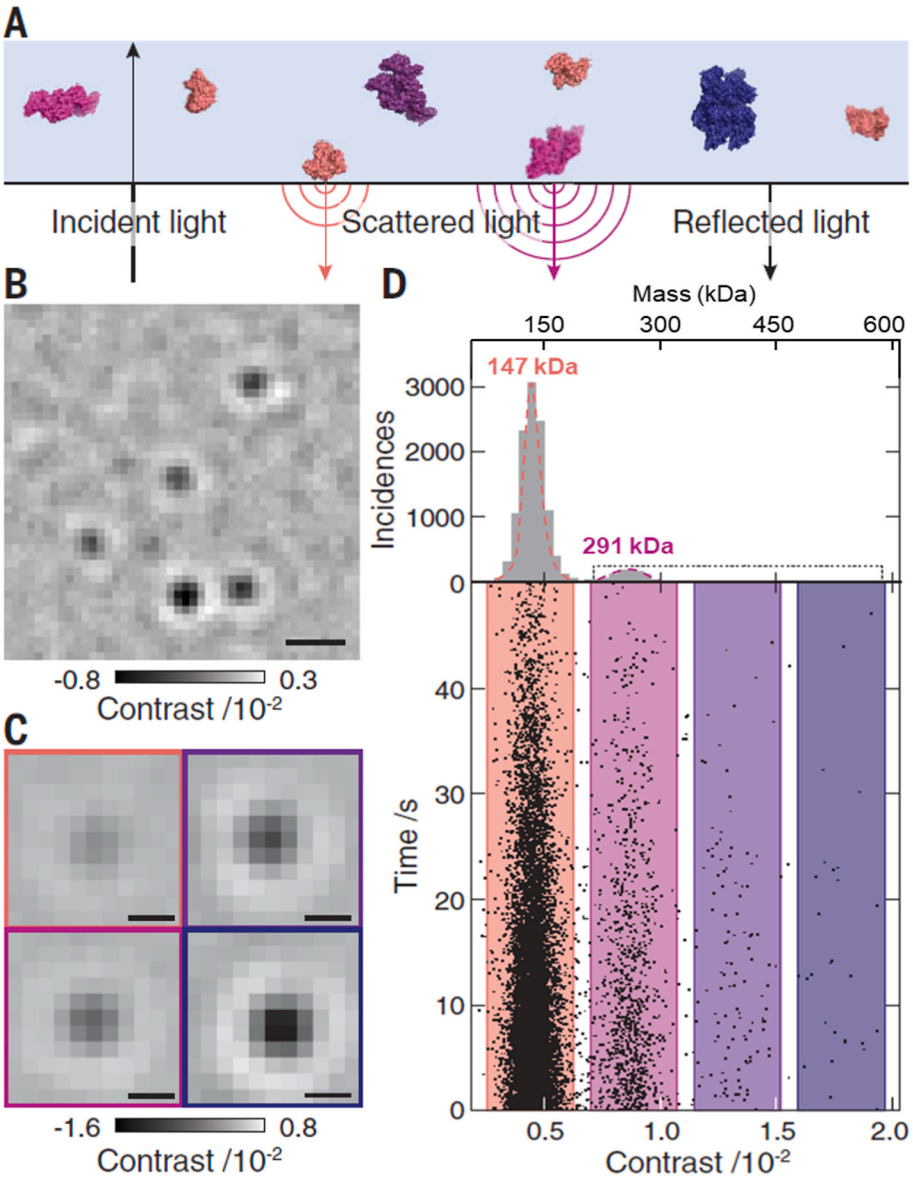

Fig. 6. Principle of single-molecule counting by mass photometry. A) Label-free single-molecule detection by imaging the interference of scattered and reflected light arising from individual landing events at a glass-water interface over time. B) Differential interferometric scattering image of the binding events. C) Representative images of molecules with different masses (larger molecules have more intense contrasts). D) Scatter plot of single-molecule contrasts and resulting mass distribution (indicative mass values). Adapted from Ref. [84].

heterogeneity and dynamics of protein-protein interactions involving therapeutic mAbs. The light-scattering method is a reliable technique for the in-solution analysis of protein complexes and can fill the gaps of others characterization techniques. In particular, the possibility to characterize interactions in-solution maintains *in vivo* dynamics compared to other techniques like X-ray crystallography and cryogenic electron microscopy, implying static analytes for the analysis. Still, some limitations of MP have also been highlighted such as the low mass resolving power. Moreover, MP has not been yet used for the analysis of protein complexation in biological matrices. Nevertheless, further technological and methodological improvements concerning MP should be expected in the near future in order to place it as an essential tool to understand mAbs complex interactions.

4. Mass spectrometry based analysis

4.1. Sample preparation

The analysis of therapeutic mAbs in biological samples also integrates an additional complexity. Indeed, it represents the analysis of a therapeutic IgG, present at nanomolar range concentration, in an extremely complex matrix containing up to 120 μM of natural IgGs in the case of serum, and several thousands of different molecules and biomolecules [93]. Therefore, sample preparation becomes a pivotal element in order to achieve a robust detection and accurate quantification of mAbs and ADA. Sample preparation should take into account the complexity of the biological sample, and the characteristics of the subsequent analysis regarding injection volume and mass analyzer instrumental sensitivity for instance. Usually, an initial step is incorporated to reduce the sample complexity using depletion or isolate therapeutic mAbs from the matrix (Fig. 8).

Sample depletion represents the removal of highly abundant proteins

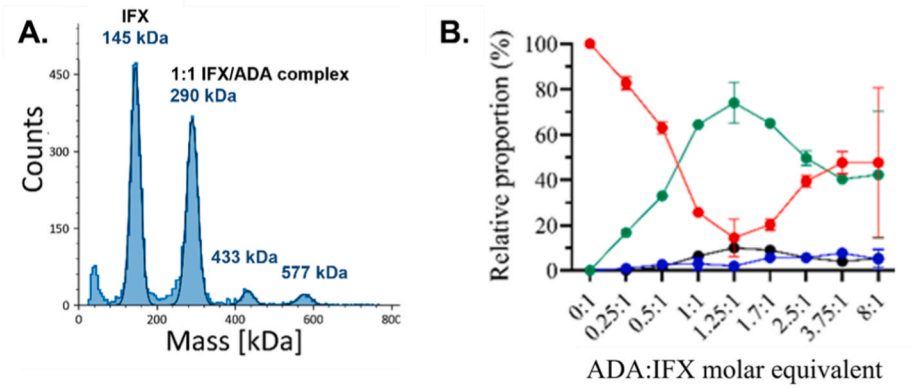

Fig. 7. A) MP analysis of the complex formation of infliximab (IFX) and ADA in PBS. B) Evolution of species relative proportion for different IFX:ADA molar equivalents using MP in PBS. Red dots: Free mAbs (signal at 145 kDa), green dots: 1:1 stoichiometry (signal at 290 kDa), blue dots: 1:2 or 2:1 stoichiometry (signal at 433 kDa) and black dots: 2:2 stoichiometry (signal at 577 kDa). Adapted from Ref. [92].

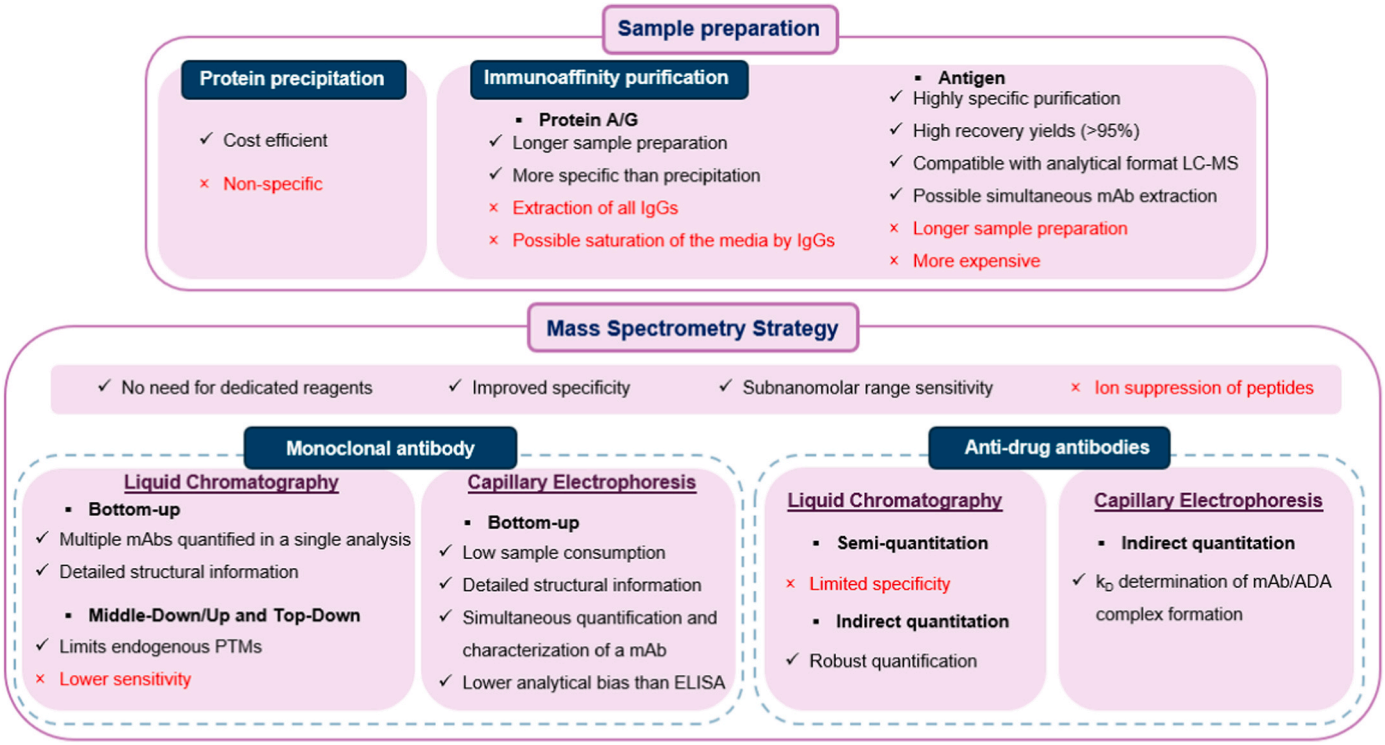

Fig. 8. Overview diagram of the benefits and drawbacks of the mass spectrometry strategy for mAb and ADA analysis.

composing the sample. Different protocols are available to perform albumin removal of biological samples, they often rely on albumin immune-affinity using short column formats [94,95]. Protein precipitation can also be used to reduce the complexity of the sample. Indeed, it represents a cost-effective way to remove a large variety of small organic compounds and other contaminants potentially present in serum or plasma. Addition of organic solvent causes a brutal reduction of the dielectric constant of the sample, disrupting the hydrophobic interactions of proteins leading to their precipitation [96]. Nguyen *et al* used only organic solvent protein precipitation for sample preparation in order to perform the absolute quantification of rendomab-B1 in serum and plasma samples compatible with pharmacokinetic studies [97]. Protein precipitation is particularly simple to perform but often does not provide specificity. Therefore, various developments were made in order to induce partial specificity toward the precipitation of mAbs, in particular using different types of anionic polyelectrolytes [98]. The implementation of polyanetholesulfonic acid as a precipitation agent allowed to achieve highly effective extraction in model biological matrices [99]. More recently, Schäfer *et al* described the use of ammonium sulphate for the precipitation of different types of mAbs. They could be consequently quantified using a bottom-up approach by analytical format LC-MS/MS using a triple quadrupole instrumentation [100].

Sample preparation can also be designed to perform the extraction of therapeutic mAbs from biological matrices, usually with minimized steps while providing medium to high specificity. Immunoaffinity purification (IP) is particularly adapted for the isolation of therapeutic mAbs from biological samples. To perform IP, ligands interacting with the protein of interest are immobilized on a static solid media. Different reactions can be implemented to allow the immobilization of ligands on the surface of adequately prepared media like porous resins, agarose or ferromagnetic beads [101]. Different proteins, interacting with the F_c

region are commonly used to perform the extraction of IgGs from biological samples. Protein A immobilized on sepharose resin was used to perform IP of trastuzumab and bevacizumab from human plasma followed by nanoflow LC-MS/MS for their bottom-up quantitative analysis [102]. Similarly, protein G immobilized on ferromagnetic beads was used for the IP extraction of different therapeutic mAbs from serum samples followed by LC-MS/MS analysis using a quantitative bottom-up strategy [103,104]. Protein A and protein G represent versatile partners for the IP of different types of therapeutic mAbs [105]. They provide efficient purification from abundant proteins. However, after purification, samples will still contain a large number of natural IgGs co-extracted from the matrix. Their presence at high concentrations could potentially result in the saturation of the IP media, preventing the optimal extraction of the mAbs of interest, and generate interferences during MS analysis.

To address limitations of protein A/G IP-based extraction, ligands specifically interacting with the therapeutic mAb of interest can be incorporated to the surface of the IP media. Purified antigens are used which allows to drastically improve extraction specificity and prevents unforeseen saturation of the extraction media. Moreover, it reduces the probability of MS signal interferences originating from other proteins. Jourdil *et al* reported the immobilization of ligands in pipet tips for IP extraction step to immobilize TNF-α in order to obtain the purification of infliximab from plasma [106]. Similarly, streptavidin-coated 96 wells plates was used to immobilize biotinylated TNF-α for the IP extraction of infliximab followed by LC-MS/MS absolute quantification [107]. Also, Reinert *et al* described the immobilization of TNF-α on the surface of streptavidin-coated magnetic beads to perform the extraction of infliximab and adalimumab in human serum. They demonstrated infliximab extraction yield systematically superior to 95 % using indirect ELISA quantification [108]. The usage of immobilized antigen IP delivered outstanding recovery yields even for complex biological samples. The implementation of antigen-based IP has permitted the quantification of mAbs in biological samples using analytical format instrumentation readily available and MS instrumentation incorporating different types of mass analyzers [100]. Other ligands were recently explored for the specific IP extraction of mAbs in biological samples. Thus, Sun *et al* developed the implementation of aptamers immobilized on streptavidin agarose beads for the purification of trastuzumab from serum samples [109].

The characterization and quantification of mAbs using MS is conventionally performed by the intermediate of peptide-centric analysis. As a consequence, proteolytic digestion is realised consequently to sample purification detailed previously. Similarly to quantitative proteomics analysis, trypsin remains the preferred enzyme to perform the digestion [110]. Indeed, trypsin exhibits a good activity capable to achieve high digestion yield after a few hours of incubation. In addition, generating peptides systematically having C-terminal basic residues is favourable to protonation during the ESI ionization process which guarantees optimal MS signal sensitivity [111].

4.2. Liquid chromatography

Liquid chromatography hyphenated to mass spectrometry (LC-MS) demonstrated to be particularly adapted for sensitive, highly specific and robust analysis of biomolecules in complex matrices. Concerning mAbs analysis in biological samples, peptide centric quantification derived from bottom-up proteomic strategy is commonly used. Peptides obtained from proteolytic digestion can be conveniently separated using standard reverse phase columns to achieve their sequential elution to MS instrumentation (Fig. 8). To ensure optimal specificity, quantification peptides are selected based on their amino acid sequence singularity screened on databases, and absence of potential PTMs [112]. Therefore, selected peptides are commonly located in the F_{ab} domain of mAbs [113]. LC-MS/MS analysis of peptide mixtures can be performed using multiple reaction monitoring (MRM) or selected reaction monitoring (SRM). In part due to the extensive sample preparation required, a stable-isotope labeled (SIL) mAb is used as an internal standard.

Quantification using LC-MS/MS analysis was developed for different types of mAbs present in various biological matrices including plasma [103], serum [114], tissue [115], cerebrospinal [116] and nasal lining fluid [117]. Ligand binding assays (LBA) like ELISA are typically dependent of specific anti-idiotype reagents that may not be always available for newer mAbs. Therefore, another advantage offered by LC-MS/MS analysis is the possibility to develop the quantification for a variety of mAbs using minimal method adaptation. For instance, El Amrani *et al* described a conventional methodology for the development of LC-MS/MS quantification of mAbs [118]. Thus, LC-MS/MS quantification could be achieved for eleven different mAbs using the same sample preparation [115]. The versatility provided by MS analysis is also a major benefit for the quantification of mAbs using concomitantly in a treatment. For example, trastuzumab and pertuzumab used in bi-therapies could be quantified in serum sample in a single analysis using LC-MS/MS [119]. Also, infliximab and adalimumab are both targeting TNF-α, therefore a common LC-MS/MS method could be developed for the quantification of the two mAbs in plasma samples. The method could be used without prior information regarding the type of mAb product present in the sample [120]. Quantification of infliximab and adalimumab in serum sample from Crohn's disease patients allowed to identify a cut-off concentration potentially associated with remission [121,122]. Using a similar approach, absolute quantification of mAbs REGN10933 and REGN10987 used for the treatment of SARS-CoV-2 could be demonstrated in serum samples using LC-MS/MS [114]. LC-MS/MS analysis could be implemented for the simultaneous quantification of seven different mAbs used in the treatment of cancer in plasma sample. Results showed similar performance for the different mAbs quantification compared to ELISA reference methods [123]. Thereby, LC-MS/MS methodologies were recently reported to be able to achieve performances a minimum on par with ELISA concerning accuracy and sensitivity [124]. However, MS/MS data have provided additional specificity. Almost thirty different mAbs were successfully quantified using LC-MS/MS in biological matrices (Table 1), sometimes preceding the availability of the anti-idiotype of the mAb necessary for LBA development [117]. LBA like ELISA present some limitations due to occasional interferences originating from the biological matrices or with ADA interfering with the quantification causing epitope binding [125–127].

On contrary, LC-MS/MS quantification was not subjected to significant matrix effects or the presence of ADAs, however the incorporation of an internal standard is mandatory to obtain a suitable quantification [143]. LC-MS/MS analysis also showed reliable quantification of IgG4, that can be problematic due to the occurrence of F_{ab} arm exchanges. Indeed, because the quantification is strictly based on specific peptides, their quantity remains equivalent even in case of arm exchanges [116]. In contrast, LBA can be significantly impacted by IgG4 F_{ab} arm exchange leading to important quantification biases [144]. MS based quantification of mAbs is linked to the availability of a protein specific peptide consequently to proteolytic digestion. However, ionization efficiency is dependent on the chemical nature of the analytes which significantly impacts signal intensity and sensitivity. Therefore, the performances of the LC-MS/MS may be affected depending on the type of mAbs, even though the inherent sensitivity of MS instrumentation provides relevant quantification for treated patients.

One of the trends regarding recent analytical developments for the quantification of mAbs using LC-MS(/MS) analysis is driven by using other methodologies as alternative to peptide centric experiments. Thus, a middle-up analysis could be developed for the quantification of rituximab using LC-MS analysis [132]. Using a middle-up approach, the μLC-MS quantification of eculizumab could also be developed demonstrating similar performances [134]. The quantification of adalimumab was also developed using 2D-LC-MS analysis. Heavy chains and light chains separation was achieved by cation exchange followed by reverse

Table 1
Mass spectrometry quantification strategy depending on the mAb or ADA quantified and the matrix.

Mab or ADA quantified	Sample volume of matrix	Purification method	Internal standard	Quantification method	LOQ/LLOQ	Reference
IFX	125 µL (human serum)	antibody precipitation (ammonium sulphate)	Horse IgG/ SIL-peptides	2 peptides (SRM)	1.0 µg mL^{-1}	[128]
	5 µL (human serum or plasma)	filter plate	SIL-peptides	2 peptides (MRM)	0.72 µg mL^{-1}	[124]
	2–100 µL (human serum)	immunoaffinity (TNF-α coated plate or beads)	SIL-IFX	1 peptide (SRM or MS signal)	0.5 µg mL^{-1} 0.22 µg mL	[107, 129]
Natalizumab	Human serum and CSF	–	Murine Ig	3 peptides (MRM)	1 µg mL^{-1}	[116]
ADM	10 µL (human plasma)	non-antibody serum proteins binding support	SIL-ADM	Light chain (MS signal)	1 µg mL^{-1}	[130]
Toripalimab	35 µL (human plasma)	protein precipitation (centrifugation)	SIL-peptide	1 peptide (MRM)	5.03 µg mL^{-1}	[131]
RTX	20 µL (human serum)	non-antibody serum protein binding support	VDZ	Light chain (MS signal)	9.7 µg mL^{-1}	[132]
CTX	50 µL (human plasma)	protein precipitation (methanol)	SIL-peptides	2 peptides (SRM)	1 µg mL^{-1}	[133]
TZM	50 µL (human serum)	protein precipitation (methanol)	–	1 peptide (MRM)	0.05 fmol $µL^{-1}$	[113]
TZM	100 µL (human serum)	immunoaffinity (aptamer coated beads)	SIL-peptide	1 peptide (MRM)	0.5 µg mL^{-1}	[109]
ECZ	50 µL (human serum)	immunoaffinity (spin Column IgG4 affinity)	PBZ	Light chain (MS signal)	5 µg mL^{-1}	[134]
USM	35 µL (human serum)	cation exchange plate	Murine IgG	1 peptide (MRM)	0.4 µg mL^{-1}	[135]
BVZ	2,25 µL (human plasma)	size Exclusion Spin Column	SIL-peptide	1 peptide (MRM)	1.8 µg mL^{-1}	[112]
DNX	10 µL (human plasma)	antibody precipitation (ammonium sulphate)	SIL-peptide	1 peptide (MRM)	1 µg mL^{-1}	[136]
Canakimumab	20 µL (human plasma)	immunoaffinity (protein G coated plate)	CTX or RTX	1 peptide (MRM)	0.1 µg mL^{-1}	[111]
HDIT101 antibody	25 µL (human plasma)	immunoaffinity (protein A resin)	SIL-peptide	1 peptide (MRM)	20 µg mL^{-1}	[110]
TZM or BVZ	10 µL TZM 5 µL BVZ	immunoaffinity (protein G coated beads)	TCZ	1 peptide (MRM)	2,5 µg mL^{-1} TZM 10 µg mL^{-1} BVZ	[104, 137]
TXG and CGV	20 µL (human serum or swab for NLF)	immunoaffinity (protein A/G coated beads)	SIL-peptides	1 peptide/mAb (MRM)	0.3 µg mL^{-1} (serum) 5 ng mL^{-1} (NLF)	[117]
REGN10933 and REGN10987	5 µL (human serum)	–	SIL-peptides	1 peptide/mAb (SRM)	20 µg mL^{-1} REGN10933 10 µg mL^{-1} REGN10987	[114]
ADM and IFX	<50 µL (human plasma)	immunoaffinity (TNF-α coated tips or plate)	SIL-IFX and SIL-ADM	1 peptide/mAb (MRM or SRM)	1 µg mL^{-1}	[120, 138]
TZM and PTZ	5–10 µL (human serum)	immunoaffinity (protein A coated beads or resin)	SIL-mAb or SIL-peptide	1 peptide/mAb (MRM)	0.25 µg mL^{-1} TZM 0.3 µg mL^{-1} PTZ	[119, 139]
RTX and ECZ	20 µL (human plasma)	immunoaffinity (protein G coated plate)	SIL-ADM	1 peptide/mAb (MRM)	1 µg mL^{-1} RTX 5 µg mL^{-1} ECZ	[47]
BVZ, TZM, TCZ and NVM	DBS (human blood)	immunoaffinity (protein G coated beads)	PBZ	1 peptide/mAb (MRM)	5 µg mL^{-1} BVZ - 10 µg mL^{-1} TZM - 13 µg mL^{-1} TCZ - 8 µg mL^{-1} NVM (LOQ)	[103]
IFX, RTX, CTX, DPL, DNX, VDZ and EMZ	10 µL (human serum or plasma)	antibody precipitation (ammonium sulphate)	SIL-peptides	1 peptide/mAb (SRM)	1 µg mL^{-1} IFX DNX VDZ 2 µg mL^{-1} CTX DPL 4 µg mL^{-1} RTX EMZ	[118]
BVZ, CTX, PBZ, RTX, TZM, IPLC and NVM	20 µL (human plasma)	immunoaffinity (affinity resin to IgG)	SIL of each mAb	1 peptide/mAb (MRM)	2 µg mL^{-1}	[123]
ADM, CTX, IFX, RTX, TZM, SCK and TCZ	20 µL (human plasma)	immunoaffinity (protein G resin)	SIL-ADM	1 peptide/mAb (MRM)	1 µg mL^{-1} RTX 5 µg mL^{-1} ADM CTX IFX TZM TCZ SCK	[122]
IFX, CTX, RTX, PTZ, ECZ, BVZ, DVL, NVM, VDZ, OCZ, USM	50 µL (human serum)	protein precipitation (caprylic acid)	SIL-peptides	1 peptide/mAb (MRM)	12.8 µg mL^{-1} PBZ - 20 µg mL^{-1} ECZ - 4 µg mL^{-1} IFX CTX RTX BVZ NVM VDZ OCZ USM DVL(LLOQs)	[115]
NADA against IFX	5 µL (human plasma)	immunoaffinity (TNF-α coated plate)	SIL-ADM	1 peptide (SRM)	3–96 µg mL^{-1}	[140]
ADA (IgG1-4, IgM, IgE, IgA1, IgA2)	95 µL (human plasma)	immunoaffinity (protein Z beads/ mouse mAb directed against human IgG coated beads)	SIL-peptides	1 peptide/isotype (MRM)	0.5 µg mL^{-1} IgG1 - 0.25 µg mL^{-1} IgM - 0.1 µg mL^{-1} IgE IgG4 IGA1 IgA2	[141]
ADA against IFX	5 µL (human plasma)	immunoaffinity (protein A coated beads)	SIL-IFX F (ab')2	1 peptide (SRM)	0.1 µg mL^{-1}	[142]

Abbreviations: ADM: Adalimumab; BVZ: Bevacizumab; CGV: Cilgavimab; CSF: Cerebrospinal fluid; CTX: Cetuximab; DBS: Dried blood spot; DNX: Dinutuximab; DVL: Durvalumab; DPL: Dupilumab; ECZ: Eculizumab; EMZ: Emicizumab; IFX: Infliximab; IPL: Ipilimumab; NLF: Nasal Lining Fluid; NVM: Nivolumab; OCZ: Ocrelizumab; PBZ: Pembrolizumab; PTZ: Pertuzumab; RTX: Rituximab; SCK: secukinumab; TCZ: Tocilizumab; TXG: Tixagevimab; TZM: Trastuzumab; USM: Ustekinumab; VDZ: Vedolizumab.

phase chromatography [130]. The second dimension of separation was needed only to place the heavy and light chains in solvent conditions compatible with ESI ionization. In most cases only the signal corresponding to the light chain could be considered due to the inherent heterogeneity of heavy chains from N-glycosylations. The identification was usually based on MS measurements, therefore it required high resolution MS analysis in order to prevent signal misinterpretation and false positive. To further improve the confidence of the identification, middle-down analysis can be performed. Collision induced dissociation (CID) routinely used for peptide fragmentation cannot deliver sufficient energies to enable free chains fragmentation. Therefore electron-transfer dissociation (ETD) providing higher energies must be incorporated. For instance, adalimumab extracted using affinity purification was submitted to limited proteolysis to generate large fragments consequently analysed by nanoflow LC-MS/MS using CID/ETD fragmentation on a FT-ICR instrument. Results showed confident identifications by means of systematic sub-1 ppm mass accuracies and MS/MS sequence coverage up to 81 %. Still, sensitivity remained limited to important concentration ranging from 29 to 2436 μg mL^{-1} [145]. Limited sensitivities observed for the analysis of large fragments is explained by a lower ionization efficiency in the case of larger macromolecules, and the occurrence of ion suppression effect originating from the sample content.

The quantification of mAbs was also explored by the intermediate of intact proteins analysis using top-down strategy, in order to remove time-consuming proteolysis and limit endogenous PTMs [146]. However, at the moment lower sensitivities observed for intact proteins are still preventing the application of top-down MS analysis for the quantification of mAbs in biological samples. Nevertheless, significant improvements concerning sample preparation and MS instrumentation should be expected in the future to envisage top-down MS quantification in clinical applications. Especially, methodology compatible with native MS should be developed in order to further characterize mAbs in conditions close to its *in vivo* state. Because the problematic regarding biological sample complexity will still be present, it appears essential to develop sample purification enabling to maintain purified mAbs in their native conditions. Addressing this analytical challenge will provide the possibility to explore further the evolution of therapeutic mAbs after their administration for example.

Concerning ADAs which represents a trending aspect of LC-MS(/MS) development for clinical analysis, dedicated methodologies were designed in order to enable ADAs quantification using MS based analysis. Semi-quantitation of ADA was recently developed using affinity-based purification of ADAs followed by proteolytic digestion and LC-MS/MS analysis. ADAs quantification was achieved by the intermediate of peptides from the IgG constant domain as they are naturally produced IgGs. This methodology was used for the quantification of ADAs in plasma, showing a minimal concentration of 0.5 μg mL^{-1} [141]. The specificity of this type of analytical strategy may be limited. As a consequence, recent developments described indirect quantitation of ADA based on the immunocapture of IgGs in sera by adding mAb F_{ab} fragment. The F_{ab} fragments involved in interactions are quantified in order to deduce the concentration of ADAs in plasma samples [142]. Another study described the indirect quantification of ADAs based on the proportion of mAbs neutralized after addition of a fixed concentration of infliximab. After equilibrium, the fraction of infliximab not forming complexes with ADAs, was extracted using immobilized TNF-α. Results showed the possibility to achieve a robust quantification of ADAs for concentration as low as 1 μg mL^{-1} and ADA quantification in agreement with ELISA [140].

Contrary to LBA and molecular interaction analysis, MS analysis is able to provide structural information regarding mAbs. Thus, LC-MS/MS was extensively used for the characterization of major PTMs occurring on mAbs, either using bottom-up [147] or middle-up approaches [148] as part of *in vitro* stability studies. Furthermore, such feature was used to determine the level of a single Asn deamidation after administration of Anti-CRTh2 mAbs. LC-MS/MS analysis of the peptide mixture allowed to perform the relative quantification of deamidation considering the affected peptide over a period of 12 weeks [149]. Using a similar methodology, precise *in vivo* monitoring of three modifications affecting an asparagine located in the CDR domain in the case of trastuzumab could be performed. The specific modification of this residue characterized by LC-MS/MS could be correlated to a loss of trastuzumab recognition in ELISA assays, attributed to an altered affinity for the HER-2 antigen [150]. Lately, the LC-MS/MS analysis of trastuzumab *in vivo* led to the identification of amino acids prone to PTMs in regions crucial for mAbs binding, that could potentially impact its potency and affinity [151]. It is important to note that sample treatment commonly used for mAbs bottom-up analysis, may induce endogenous PTMs especially Asn deamidation. Hopefully, careful optimization of sample preparation can be realised in order to limit drastically undesired modifications of the protein. Moreover, the *in vivo* and *in vitro* comparison study clearly identified the occurrence of PTMs after administration of therapeutic mAbs [151]. Thus, the implementation of LC-MS/MS analysis is particularly interesting due to the possibility to perform highly specific and sensitive quantification. In addition, this technique can provide detailed characterization which could be used in the future to understand their impact on mAbs activity.

4.3. Capillary electrophoresis

Capillary electrophoresis (CE) enables a high-resolution separation of charged species in solution, particularly relevant for the analysis of proteins and peptides. Thus, various CE methods could be developed for the characterization of mAbs over the different levels defining their structure [152]. Due to a relevant combination of their characteristics, CE coupled to MS has emerged as a powerful technique for therapeutic mAbs products characterization [153]. Direct CE-MS hyphenation could also be used for the study of glycoforms and charge variants composing several therapeutic mAbs products [154]. CE-MS instrumentation is experiencing major technical improvements as well, like the development of 2D-CE-MS for the detailed identification of mAbs charge variants in their intact form [155].

Simultaneous quantification and structural characterization of infliximab in serum samples using CE-MS/MS analysis could be successfully developed (Fig. 8). The analytical strategy, based on a bottom-up strategy, was composed of antigen-affinity purification and proteolytic digestion, followed by CZE-ESI-MS/MS analysis [129]. Compared to the corresponding ELISA assay, CE-MS/MS showed consistent quantification and significantly lower analytical biases (Fig. 9A). CE-MS/MS data allowed to simultaneously identify different types of PTMs including N-glycosylations, Asp deamidation and Asp isomerization. Also, a novel MS signal normalization was incorporated to data treatment in order to address any eventual endogenous PTMs generated during sample preparation. Results allowed to monitor the evolution of the glycan distribution after administration, enabling to identify fast clearance variants independently. Interestingly, the comparison of the concentration of active infliximab depending on the anteriority of the administration did not demonstrate any conclusive correlation (Fig. 9B). Whereas the residue Asn^{57}, located in the CDR regions, exhibited gradual modification depending on time since administration potentially affecting its interaction with TNF-α (Fig. 9C). Thus, they demonstrated that clinical follow-up considering exclusively free mAbs concentration may not be sufficient to fully understand the phenomenon undergone by mAbs during their residence time. The performance of the CE-MS/MS analytical method clearly demonstrated the added value advanced MS analysis can bring to study complex biological phenomenon [129]. For CE-MS/MS analytical workflow, comprehensive assessment and optimization of the sample preparation was realised in order to provide an optimal sample compatibility with both CE separation and ESI-MS/MS analysis [108]. Using a similar analytical strategy, CE-MS/MS analysis could be used to perform the biosimilarity

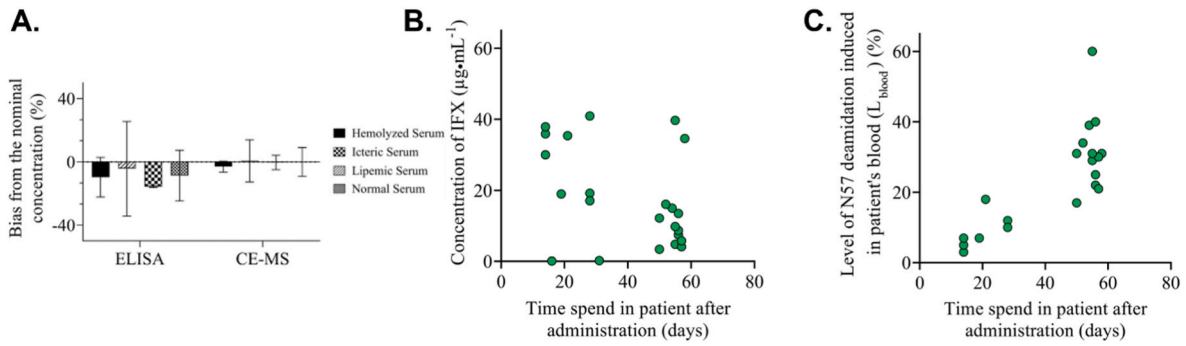

Fig. 9. A) Infliximab quantification accuracy and repeatability for different types of spiked serums measured using ELISA assay and CE-MS/MS analysis, evolution of B) infliximab concentration in patient serums and C) deamidated level of N57 with anteriority of the administration. Adapted from Ref. [129].

assessment between different infliximab products concerning *in vivo* stability. In addition, infliximab PTMs characterization was performed for serum samples from treated patients [156].

CE-MS/MS was also recently described to investigate the interaction between infliximab and a corresponding NADA. In order to determine the quantity of NADA in a serum sample, fixed quantities of internal standards SIL-infliximab and SIL-adalimumab were introduced to the sample. Afterward, affinity purification was performed followed by subsequent proteolytic digestion. CE-MS/MS signal corresponding to the two mAbs was used in order to determine the proportion of neutralized SIL-infliximab compared to unaffected SIL-adalimumab, and deduce the initial concentration of NADAs in the serum. Experiments allowed to determine the dissociation constant K_D of 14.4 nM between infliximab and NADAs [92]. In particular, this methodology designed to determine K_D value from CE-MS/MS data took into account the bivalence of both infliximab and NADAs due to their IgG nature. Infliximab-NADAs interaction was generated in serum samples to provide an environment similar to *in vivo* conditions. Another advantage of this methodology was the ability to investigate infliximab-NADAs interaction without any information regarding the amino acid sequence of neutralizing ADAs [92]. Considering the excellent characteristics of CE-MS/MS analysis, further developments could be envisaged in the near future to achieve the simultaneous quantification of therapeutic mAbs and corresponding NADAs in biological samples.

5. Challenges summary and future prospects

Considering the complexes phenomena undergone by mAbs, it appears crucial to investigate their outcome after administration in the context of treatment follow-up and/or clinical applications. Similarly, the so-far unpredictable expression of ADA and their implication urges the implementation of relevant approaches for their detection and characterization. Different analytical methodologies can be used depending on the nature of the information required (e.g. quantification, structural modifications, interactions characterization) and the clinical context.

LBA, with ELISA as the most common alternative, provide mainly quantitative information of mAbs and ADA in biological samples with a cost-effective and rapid analysis with commonly available instrumentation. However, CLIA sensitivity, compared to ELISA, appeared particularly relevant for the early identification of adverse immune response in the form of ADA expression. Thus, automated instrumentations were recently developed, opening the wide scale use of CLIA for routine quantification of mAbs and ADAs. Even though LBA remains mainly for quantitative analysis, ELISA can be used to measure molecular interactions with complex experiments and dedicated assays. Therefore, SPR represents the method of choice to study mAbs interactions with other proteins like the targeted antigen, receptors and even ADAs. SPR is capable to measure in a precise manner kinetic and equilibrium binding constants to study the affinity of mAbs with different types of proteins in a versatile manner. However, various parameters need to be considered for the implementation of SPR which may be relatively complex especially for neophytes. The recent introduction of MP analysis represents an excellent opportunity for the characterization of mAbs molecular interactions. Compared to other techniques like SPR or FC, it represents an easy-to-handle technique that does not require protein immobilization for the analysis. Thus, MP has been used to study complex formation between mAbs and antigens or ADAs, while providing precise information regarding the stoichiometries in comparison to SPR. Due to the recent introduction of MP, further developments taking full profit of the potential of this technique are expected in the future to improve its applicability to study protein-protein interactions.

MS emerged over the last years as a pivotal tool to study the evolution of mAbs after administration. Indeed, in the context of quantification in biological samples, MS showed its ability to prevent signal interferences from other proteins that was observed in ELISA and to limit drastically the occurrence of matrix effects. In complement, MS analysis prior hyphenated to a chromatographic or electrophoretic separation demonstrated the possibility to obtain primary structure characterization like the identification of PTMs. Therefore, MS based methodologies may provide a novel perspective regarding the structural evolution of mAbs after administration. Nonetheless, the implementation of MS for the analysis of mAbs and ADAs in biological samples may be restricted at the moment to fundamental and investigational clinical applications due to the extensive sample treatment required. To further envisage MS analysis for the routine follow-up of patients, methodological developments should be performed in the future to reduce the complexity and time necessary for the analysis. Moreover, native MS represents one of the most advanced types of MS experiments, with demanding experimental conditions. Native MS in biological samples could help tremendously to improve the knowledge concerning protein-protein interactions involving therapeutic mAbs following their administration.

CRediT authorship contribution statement

Lola Alez-Martin: Writing – review & editing, Visualization, Investigation, Writing – original draft, Methodology. **Emilie Hirschler:** Writing – original draft, Investigation. **Pascal Houzé:** Writing – review & editing. **Noëlle Potier:** Writing – review & editing. **Nathalie Mignet:** Writing – review & editing. **Emmanuelle Leize-Wagner:** Writing – review & editing. **Yannis-Nicolas François:** Writing – original draft, Writing – review & editing, Conceptualization. **Rabah Gahoual:** Writing – review & editing, Methodology, Conceptualization, Writing – original draft, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

[1] S. Crescioli, H. Kaplon, A. Chenoweth, L. Wang, J. Visweswaraiah, J.M. Reichert, Antibodies to watch in 2024, mAbs 16 (2024) 2297450, https://doi.org/10.1080/19420862.2023.2297450.

[2] F. Breedveld, Therapeutic monoclonal antibodies, Lancet 355 (2000) 735–740, https://doi.org/10.1016/S0140-6736(00)01034-5.

[3] U. Hafeez, H.K. Gan, A.M. Scott, Monoclonal antibodies as immunomodulatory therapy against cancer and autoimmune diseases, Curr. Opin. Pharmacol. 41 (2018) 114–121, https://doi.org/10.1016/j.coph.2018.05.010.

[4] J. Nixon, P. Newbold, T. Mustelin, G.P. Anderson, R. Kolbeck, Monoclonal antibody therapy for the treatment of asthma and chronic obstructive pulmonary disease with eosinophilic inflammation, Pharmacol. Therapeut. 169 (2017) 57–77, https://doi.org/10.1016/j.pharmthera.2016.10.016.

[5] A. Beck, L. Goetsch, C. Dumontet, N. Corvaïa, Strategies and challenges for the next generation of antibody–drug conjugates, Nat. Rev. Drug Discov. 16 (2017) 315–337, https://doi.org/10.1038/nrd.2016.268.

[6] K.L. Gill, K.K. Machavaram, R.H. Rose, M. Chetty, Potential sources of inter-subject variability in monoclonal antibody pharmacokinetics, Clin. Pharmacokinet. 55 (2016) 789–805, https://doi.org/10.1007/s40262-015-0361-4.

[7] S.C. Sasson, L.E. Wilkins, R.A. Watson, C. Jolly, O. Brain, P. Klenerman, A. Olsson-Brown, B.P. Fairfax, Identification of neutralising pembrolizumab anti-drug antibodies in patients with melanoma, Sci. Rep. 11 (2021) 19253, https://doi.org/10.1038/s41598-021-98700-7.

[8] S. Fekete, D. Guillarme, P. Sandra, K. Sandra, Chromatographic, electrophoretic, and mass spectrometric methods for the analytical characterization of protein biopharmaceuticals, Anal. Chem. 88 (2016) 480–507, https://doi.org/10.1021/acs.analchem.5b04561.

[9] J.T. Ryman, B. Meibohm, Pharmacokinetics of monoclonal antibodies, CPT Pharmacometrics Syst. Pharmacol. 6 (2017) 576–588, https://doi.org/10.1002/psp4.12224.

[10] M.L. Chiu, D.R. Goulet, A. Teplyakov, G.L. Gilliland, Antibody structure and function: the basis for engineering therapeutics, Antibodies 8 (2019) 55, https://doi.org/10.3390/antib8040055.

[11] Y. Kronimus, R. Dodel, S.P. Galuska, S. Neumann, IgG Fc N-glycosylation: alterations in neurologic diseases and potential therapeutic target? J. Autoimmun. 96 (2019) 14–23, https://doi.org/10.1016/j.jaut.2018.10.006.

[12] M. Kiyoshi, K. Tsumoto, A. Ishii-Watabe, J.M.M. Caaveiro, Glycosylation of IgG-Fc: a molecular perspective, Int. Immunol. 29 (2017) 311–317, https://doi.org/10.1093/intimm/dxx038.

[13] B. Wei, K. Berning, C. Quan, Y.T. Zhang, Glycation of antibodies: modification, methods and potential effects on biological functions, mAbs 9 (2017) 586–594, https://doi.org/10.1080/19420862.2017.1300214.

[14] S. Bournazos, A. Gupta, J.V. Ravetch, The role of IgG Fc receptors in antibody-dependent enhancement,, Nat. Rev. Immunol. 20 (2020) 633–643, https://doi.org/10.1038/s41577-020-00410-0.

[15] H. Liu, G. Gaza-Bulseco, D. Faldu, C. Chumsae, J. Sun, Heterogeneity of monoclonal antibodies, J. Pharmaceut. Sci. 97 (2008) 2426–2447, https://doi.org/10.1002/jps.21180.

[16] W. Wang, S. Singh, D.L. Zeng, K. King, S. Nema, Antibody structure, instability, and formulation, J. Pharmaceut. Sci. 96 (2007) 1–26, https://doi.org/10.1002/jps.20727.

[17] L. Liu, Pharmacokinetics of monoclonal antibodies and Fc-fusion proteins, Protein Cell 9 (2018) 15–32, https://doi.org/10.1007/s13238-017-0408-4.

[18] Y. Yan, H. Wei, Y. Fu, S. Jusuf, M. Zeng, R. Ludwig, S.R. Krystek, G. Chen, L. Tao, T.K. Das, Isomerization and oxidation in the complementarity-determining regions of a monoclonal antibody: a study of the modification–structure–function correlations by hydrogen–deuterium exchange mass spectrometry, Anal. Chem. 88 (2016) 2041–2050, https://doi.org/10.1021/acs.analchem.5b02800.

[19] A.M. Alsamil, T.J. Giezen, T.C. Egberts, H.G. Leufkens, H. Gardarsdottir, Type and extent of information on (potentially critical) quality attributes described in European public assessment reports for adalimumab biosimilars, Pharmaceuticals 14 (2021) 189, https://doi.org/10.3390/ph14030189.

[20] A. Ambrogelly, S. Gozo, A. Katiyar, S. Dellatore, Y. Kune, R. Bhat, J. Sun, N. Li, D. Wang, C. Nowak, A. Neill, G. Ponniah, C. King, B. Mason, A. Beck, H. Liu, Analytical comparability study of recombinant monoclonal antibody therapeutics, mAbs 10 (2018) 513–538, https://doi.org/10.1080/19420862.2018.1438797.

[21] F. Higel, A. Seidl, F. Sörgel, W. Friess, N-glycosylation heterogeneity and the influence on structure, function and pharmacokinetics of monoclonal antibodies and Fc fusion proteins, Eur. J. Pharm. Biopharm. 100 (2016) 94–100, https://doi.org/10.1016/j.ejpb.2016.01.005.

[22] C.K. Imamura, Therapeutic drug monitoring of monoclonal antibodies: applicability based on their pharmacokinetic properties, Drug Metabol. Pharmacokinet. 34 (2019) 14–18, https://doi.org/10.1016/j.dmpk.2018.11.003.

[23] T.T. Hansel, H. Kropshofer, T. Singer, J.A. Mitchell, A.J.T. George, The safety and side effects of monoclonal antibodies,, Nat. Rev. Drug Discov. 9 (2010) 325–338, https://doi.org/10.1038/nrd3003.

[24] P.A. Van Schouwenburg, C.L. Krieckaert, T. Rispens, L. Aarden, G.J. Wolbink, D. Wouters, Long-term measurement of anti-adalimumab using pH-shift-anti-idiotype antigen binding test shows predictive value and transient antibody formation, Ann. Rheum. Dis. 72 (2013) 1680–1686, https://doi.org/10.1136/annrheumdis-2012-202407.

[25] F.A. Harding, M.M. Stickler, J. Razo, R. DuBridge, The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions, mAbs 2 (2010) 256–265, https://doi.org/10.4161/mabs.2.3.11641.

[26] M. Krishna, S.G. Nadler, Immunogenicity to biotherapeutics – the role of anti-drug immune complexes, Front. Immunol. 7 (2016), https://doi.org/10.3389/fimmu.2016.00021.

[27] S. Bitoun, S. Hässler, D. Ternant, N. Szely, A. Gleizes, C. Richez, M. Soubrier, J. Avouac, O. Brocq, J. Sellam, N. De Vries, T.W.J. Huizinga, E.C. Jury, J. J. Manson, C. Mauri, A. Matucci, S. Hacein Bey Abina, D. Mulleman, M. Pallardy, P. Broët, X. Mariette, F. Abirisk Consortium, P. Berenbaum, P. Dieudé, M. Bertin, C. Dougados, A. Miceli, H. Pedriger, A. Marotte, O. Cantagrel, T. Vittecoq, A. Lequere, R.-M. Saraux, J. Flipo, J.E. Sibilia, B. Gottenberg, J. Combe, D. Morel, C. Wendling, M. Verhoef, M. Van Rijswijk, A. Nurmohamed, Vultaggio, Response to biologic drugs in patients with rheumatoid arthritis and antidrug antibodies, JAMA Netw. Open 6 (2023) e2323098, https://doi.org/10.1001/jamanetworkopen.2023.23098.

[28] K.S. Nanda, A.S. Cheifetz, A.C. Moss, Impact of antibodies to infliximab on clinical outcomes and serum infliximab levels in patients with Inflammatory Bowel Disease (IBD): a meta-analysis, Am. J. Gastroenterol. 108 (2013) 40–47, https://doi.org/10.1038/ajg.2012.363.

[29] N. Vande Casteele, R. Khanna, B.G. Levesque, L. Stitt, G.Y. Zou, S. Singh, S. Lockton, S. Hauenstein, L. Ohrmund, G.R. Greenberg, P.J. Rutgeerts, A. Gils, W. J. Sandborn, S. Vermeire, B.G. Feagan, The relationship between infliximab concentrations, antibodies to infliximab and disease activity in Crohn's disease, Gut 64 (2015) 1539–1545, https://doi.org/10.1136/gutjnl-2014-307883.

[30] M.T. Frederiksen, M.A. Ainsworth, J. Brynskov, O.Ø. Thomsen, K. Bendtzen, C. Steenholdt, Antibodies Against Infliximab Are Associated with De Novo Development of Antibodies to Adalimumab and Therapeutic Failure in Infliximab-to-Adalimumab Switchers with IBD, Inflamm. Bowel Dis. 20 (2014) 1714–1721, https://doi.org/10.1097/MIB.0000000000000138.

[31] N. Vande Casteele, M.T. Abreu, S. Flier, K. Papamichael, F. Rieder, M. S. Silverberg, R. Khanna, L. Okada, L. Yang, A. Jain, A.S. Cheifetz, Patients with low drug levels or antibodies to a prior anti–tumor necrosis factor are more likely to develop antibodies to a subsequent anti–tumor necrosis factor, Clin. Gastroenterol. Hepatol. 20 (2022) 465–467, https://doi.org/10.1016/j.cgh.2021.01.006, e2.

[32] C.T. Harris, S. Cohen, Reducing immunogenicity by design: approaches to minimize immunogenicity of monoclonal antibodies, BioDrugs 38 (2024) 205–226, https://doi.org/10.1007/s40259-023-00641-2.

[33] K. Papamichael, A.S. Cheifetz, G.Y. Melmed, P.M. Irving, N. Vande Casteele, P. L. Kozuch, L.E. Raffals, L. Baidoo, B. Bressler, S.M. Devlin, J. Jones, G.G. Kaplan, M.P. Sparrow, F.S. Velayos, T. Ullman, C.A. Siegel, Appropriate therapeutic drug monitoring of biologic agents for patients with inflammatory bowel diseases, Clin. Gastroenterol. Hepatol. 17 (2019) 1655–1668, https://doi.org/10.1016/j.cgh.2019.03.037, e3.

[34] P. Galle, R.S. Finn, C.R. Mitchell, K. Ndirangu, Z. Ramji, G.S. Redhead, D. J. Pinato, Treatment-emergent antidrug antibodies related to PD-1, PD-L1, or CTLA-4 inhibitors across tumor types: a systematic review, J. Immunother. Cancer 12 (2024) e008266, https://doi.org/10.1136/jitc-2023-008266.

[35] C. Steenholdt, J. Brynskov, O.Ø. Thomsen, L.K. Munck, J. Fallingborg, L. A. Christensen, G. Pedersen, J. Kjeldsen, B.A. Jacobsen, A.S. Oxholm, J. Kjellberg, K. Bendtzen, M.A. Ainsworth, Individualised therapy is more cost-effective than dose intensification in patients with Crohn's disease who lose response to anti-TNF treatment: a randomised, controlled trial, Gut 63 (2014) 919–927, https://doi.org/10.1136/gutjnl-2013-305279.

[36] D. Xiang, N. Li, L. Liu, H. Yu, X. Li, T. Zhao, D. Liu, X. Gong, Development and validation of enzyme-linked immunosorbent assays for the measurement of infliximab and anti-drug antibody levels, Heliyon 9 (2023) e21858, https://doi.org/10.1016/j.heliyon.2023.e21858.

[37] E. Klenske, L. Osaba, D. Nagore, T. Rath, M.F. Neurath, R. Atreya, Drug levels in the maternal serum, cord blood and breast milk of a ustekinumab-treated patient with Crohn's disease, J. Crohn's and Colitis 13 (2019) 267–269, https://doi.org/10.1093/ecco-jcc/jjy153.

[38] K. Suh, I. Kyei, D.S. Hage, Approaches for the detection and analysis of anti-drug antibodies to biopharmaceuticals: a review, J. Separ. Sci. 45 (2022) 2077–2092, https://doi.org/10.1002/jssc.202200112.

[39] X. Yang, K. Siradze, G. Sperinde, A. Arjomandi, S. Fischer, Evaluation of multiple immunoassay formats for detection of anti-drug antibodies to zinpentraxin alfa, J. Immunol. Methods 522 (2023) 113573, https://doi.org/10.1016/j.jim.2023.113573.

[40] S. Atiqi, F. Hooijberg, F.C. Loeff, T. Rispens, G.J. Wolbink, Immunogenicity of TNF-Inhibitors, front, Immunol. Ser. 11 (2020), https://doi.org/10.3389/fimmu.2020.00312.

[41] M.A. Partridge, S. Purushothama, C. Elango, Y. Lu, Emerging Technologies, Generic, Assays for the detection of anti-drug antibodies, J Immunol Res 2016 (2016) 6262383, https://doi.org/10.1155/2016/6262383.

[42] M. van der Neut Kolfschoten, J. Schuurman, M. Losen, W.K. Bleeker, P. Martínez-Martínez, E. Vermeulen, T.H. den Bleker, L. Wiegman, T. Vink, L.A. Aarden, M.

H. De Baets, J.G.J. van de Winkel, R.C. Aalberse, P.W.H.I. Parren, Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange, Science 317 (2007) 1554–1557, https://doi.org/10.1126/science.1144603.
[43] J. Du, Y. Yang, L. Zhu, S. Wang, C. Yu, C. Liu, C. Long, B. Chen, G. Xu, G. Zou, L. Wang, Method validation of a bridging immunoassay in combination with acid-dissociation and bead treatment for detection of anti-drug antibody, Heliyon 9 (2023) e13999, https://doi.org/10.1016/j.heliyon.2023.e13999.
[44] L. Yuan, C.R. Gleason, D. Stocker, L. Li, J.X. Shen, Q.C. Ji, A bridging immunogenicity assay for anti-cabiralizumab antibodies: overcoming the low assay cut point and drug tolerance challenges, Bioanalysis 13 (2021) 395–407, https://doi.org/10.4155/bio-2020-0300.
[45] A. Martínez-Feito, M. Novella-Navarro, B. Hernández-Breijo, P. Nozal, D. Peiteado, A. Villalba, L. Nuño, I. Monjo, D. Pascual-Salcedo, A. Balsa, C. Plasencia-Rodríguez, Early monitoring of anti-infliximab antibodies by drug-tolerant assay predicts later immunogenicity and drug survival in rheumatic diseases, Rheumatology (2024) kead690, https://doi.org/10.1093/rheumatology/kead690.
[46] I. Iria, R.R.G. Soares, E.J.S. Brás, V. Chu, J. Gonçalves, J.P. Conde, Accurate and rapid microfluidic ELISA to monitor Infliximab titers in patients with inflammatory bowel diseases, Analyst 147 (2022) 480–488, https://doi.org/10.1039/D1AN01810H.
[47] A. Truffot, J.-F. Jourdil, B. Seitz-Polski, P. Malvezzi, V. Brglez, F. Stanke-Labesque, E. Gautier-Veyret, Simultaneous quantification of rituximab and eculizumab in human plasma by liquid chromatography-tandem mass spectrometry and comparison with rituximab ELISA kits, Clin. Biochem. 87 (2021) 60–66, https://doi.org/10.1016/j.clinbiochem.2020.10.007.
[48] M. El Amrani, A.A.M. Donners, C.E. Hack, A.D.R. Huitema, E.M. Van Maarseveen, Six-step workflow for the quantification of therapeutic monoclonal antibodies in biological matrices with liquid chromatography mass spectrometry – a tutorial, Anal. Chim. Acta 1080 (2019) 22–34, https://doi.org/10.1016/j.aca.2019.05.076.
[49] M. Carter, J. Shieh, Chapter 15-Biochemical assays and intracellular signaling, in: M. Carter, J. Shieh (Eds.), Guide to Research Techniques in Neuroscience, second ed., Academic Press, San Diego, 2015, pp. 311–343, https://doi.org/10.1016/B978-0-12-800511-8.00015-0.
[50] M. Jani, H. Chinoy, R.B. Warren, C.E.M. Griffiths, D. Plant, B. Fu, A.W. Morgan, A.G. Wilson, J.D. Isaacs, KimmeL. Hyrich, A. Barton, clinical utility of random anti–tumor necrosis factor drug–level testing and measurement of antidrug antibodies on the long-term treatment response in rheumatoid arthritis, Arthritis Rheumatol. 67 (2015) 2011–2019, https://doi.org/10.1002/art.39169.
[51] K. Bendtzen, M. Svenson, Enzyme immunoassays and radioimmunoassays for quantification of Anti-TNF biopharmaceuticals and anti-drug antibodies, in: M. G. Tovey (Ed.), Detection and Quantification of Antibodies to Biopharmaceuticals, first ed., Wiley, 2011, pp. 81–101, https://doi.org/10.1002/9781118075685.ch5.
[52] P. Barta, J. Janousek, K. Zilkova, F. Trejtnar, In vitro evaluation of concentration, labeling effectiveness and stability for 131I-labeled radioimmunoassay ligand using real-time detection technology, J. Label. Compd. Radiopharm. 60 (2017) 80–86, https://doi.org/10.1002/jlcr.3478.
[53] K. Kumar, K. Woolum, A novel reagent for radioiodine labeling of new chemical entities (NCEs) and biomolecules, Molecules 26 (2021) 4344, https://doi.org/10.3390/molecules26144344.
[54] D. Levêque, S. Wisniewski, F. Jehl, Pharmacokinetics of therapeutic monoclonal antibodies used in oncology, Anticancer Res. 25 (2005) 2327–2343.
[55] M. Jani, J.D. Isaacs, A.W. Morgan, A.G. Wilson, D. Plant, K.L. Hyrich, H. Chinoy, A. Barton, Detection of anti-drug antibodies using a bridging ELISA compared with radioimmunoassay in adalimumab-treated rheumatoid arthritis patients with random drug levels, Rheumatology 55 (2016) 2050–2055, https://doi.org/10.1093/rheumatology/kew299.
[56] P.A. Routledge, A.D. Hutchings, Chapter 9.22-Therapeutic drug monitoring (TDM). The Immunoassay Handbook, fourth ed., Elsevier Ltd, 2013, pp. 945–962, https://doi.org/10.1016/B978-0-08-097037-0.00076-2.
[57] L. Zheng, J. Wang, Y. Wang, Z. Song, Y. Dong, Y. Yin, S.A. Eremin, M. Meng, R. Xi, A sensitive chemiluminescent immunoassay for point-of-care testing of repaglinide in natural dietary supplements and serum, Anal. Bioanal. Chem. 407 (2015) 1973–1980, https://doi.org/10.1007/s00216-015-8462-3.
[58] H. Karatani, Luminol–hydrogen peroxide–horseradish peroxidase chemiluminescence intensification by kosmotrope ammonium sulfate, Anal. Sci. 38 (2022) 613–621, https://doi.org/10.1007/s44211-022-00069-8.
[59] S. Deepa, R. Venkatesan, S. Jayalakshmi, M. Priya, S.-C. Kim, Recent advances in catalyst-enhanced luminol chemiluminescence system and its environmental and chemical applications, J. Environ. Chem. Eng. 11 (2023) 109853, https://doi.org/10.1016/j.jece.2023.109853.
[60] I.A. Darwish, N.Z. Alzoman, N.N.Y. Khalil, H.W. Darwish, Novel highly sensitive chemiluminescence immunoassay for quantitation of durvalumab using a signal enhanced horseradish peroxidase-luminol-hydrogen peroxide reaction for detection system, Talanta Open 7 (2023) 100219, https://doi.org/10.1016/j.talo.2023.100219.
[61] I.A. Darwish, M.A.H. Ali, M.S. Alsalhi, D. Zhang, A novel ultrasensitive chemiluminescence enzyme immunoassay by employment of a signal enhancement of horseradish peroxidase-luminol-hydrogen peroxide reaction for the quantitation of atezolizumab, a monoclonal antibody used for cancer immunotherapy, RSC Adv. 14 (2024) 8167–8177, https://doi.org/10.1039/D4RA00202D.
[62] M. El-Maghrabey, N. Kishikawa, S. Harada, K. Ohyama, N. Kuroda, Quinone-based antibody labeling reagent for enzyme-free chemiluminescent immunoassays. Application to avidin and biotinylated anti-rabbit IgG labeling, Biosens. Bioelectron. 160 (2020) 112215, https://doi.org/10.1016/j.bios.2020.112215.
[63] T. Ma, M. Zhang, Y. Wan, Y. Cui, L. Ma, Preparation of an acridinium ester-labeled antibody and its application in GoldMag nanoparticle-based, ultrasensitive chemiluminescence immunoassay for the detection of human epididymis protein 4, Micromachines (Basel) 8 (2017) 149, https://doi.org/10.3390/mi8050149.
[64] W.H.M. Vroemen, S.S. Agata, J.J.B.C. van Beers, J.G.M.C. Damoiseaux, Therapeutic drug monitoring of infliximab and adalimumab through concentration and anti-drug antibodies assessment; comparison of sanquin diagnostics and theradiag assays, Antibodies (Basel) 13 (2024) 73, https://doi.org/10.3390/antib13030073.
[65] A.E. Berger, A. Gleizes, L. Waeckel, X. Roblin, R. Krzysiek, S. Hacein-Bey-Abina, A. Soriano, S. Paul, Validation study of a new random-access chemiluminescence immunoassay Analyzer i-TRACK10® to monitor Infliximab and Adalimumab serum trough levels and anti-drug antibodies, Int. J. Mol. Sci. 23 (2022), https://doi.org/10.3390/ijms23179561.
[66] P.V. Beum, A.D. Kennedy, R.P. Taylor, Three new assays for rituximab based on its immunological activity or antigenic properties: analyses of sera and plasmas of RTX-Treated patients with chronic lymphocytic leukemia and other B cell lymphomas, J. Immunol. Methods 289 (2004) 97–109, https://doi.org/10.1016/j.jim.2004.03.012.
[67] R. Camacho-Sandoval, E.N. Sosa-Grande, E. González-González, A. Tenorio-Calvo, C.A. López-Morales, M. Velasco-Velázquez, L. Pavón-Romero, S.M. Pérez-Tapia, E. Medina-Rivero, Development and validation of a bioassay to evaluate binding of adalimumab to cell membrane-anchored TNFα using flow cytometry detection, J. Pharmaceut. Biomed. Anal. 155 (2018) 235–240, https://doi.org/10.1016/j.jpba.2018.03.057.
[68] T.B. Nielsen, J. Yan, M. Slarve, P. Lu, R. Li, J. Ruiz, B. Lee, E. Burk, Y. Talyansky, P. Oelschlaeger, K. Hurth, W. Win, B.M. Luna, R.A. Bonomo, B. Spellberg, Monoclonal antibody therapy against *Acinetobacter baumannii*, Infect. Immun. 89 (2021) e00162, https://doi.org/10.1128/IAI.00162-21, 21.
[69] A. Harrison, Z. Liu, S. Makweche, K. Maskell, H. Qi, G. Hale, Methods to measure the binding of therapeutic monoclonal antibodies to the human Fc receptor FcγRIII (CD16) using real time kinetic analysis and flow cytometry, J. Pharmaceut. Biomed. Anal. 63 (2012) 23–28, https://doi.org/10.1016/j.jpba.2012.01.029.
[70] E.K. Makowski, L. Wu, A.A. Desai, P.M. Tessier, Highly sensitive detection of antibody nonspecific interactions using flow cytometry, mAbs 13 (2021) 1951426, https://doi.org/10.1080/19420862.2021.1951426.
[71] K. Hoshitsuki, S. Rathod, M.J. Ramsey, L. Zhu, L.W. Moreland, C.A. Fernandez, Adalimumab immunogenicity is negatively correlated with anti-hinge antibody levels in patients with rheumatoid arthritis, J. Pharmacol. Exp. Therapeut. 375 (2020) 488–497, https://doi.org/10.1124/jpet.120.000179.
[72] R. Rich, Advances in surface plasmon resonance biosensor analysis, Curr. Opin. Biotechnol. 11 (2000) 54–61, https://doi.org/10.1016/S0958-1669(99)00054-3.
[73] X. Wang, M.M. Phan, Y. Sun, J.T. Koerber, H. Ho, Y. Chen, J. Yang, Development of an SPR-based binding assay for characterization of anti-CD20 antibodies to CD20 expressed on extracellular vesicles, Anal. Biochem. 646 (2022) 114635, https://doi.org/10.1016/j.ab.2022.114635.
[74] G. Scapin, X. Yang, W.W. Prosise, M. McCoy, P. Reichert, J.M. Johnston, R. S. Kashi, C. Strickland, Structure of full-length human anti-PD1 therapeutic IgG4 antibody pembrolizumab, Nat. Struct. Mol. Biol. 22 (2015) 953–958, https://doi.org/10.1038/nsmb.3129.
[75] O. Montacir, H. Montacir, M. Eravci, A. Springer, S. Hinderlich, A. Saadati, M. K. Parr, Comparability study of Rituximab originator and follow-on biopharmaceutical, J. Pharmaceut. Biomed. Anal. 140 (2017) 239–251, https://doi.org/10.1016/j.jpba.2017.03.029.
[76] F. Real-Fernández, R. Cimaz, G. Rossi, G. Simonini, T. Giani, I. Pagnini, A. M. Papini, P. Rovero, Surface plasmon resonance-based methodology for anti-adalimumab antibody identification and kinetic characterization, Anal. Bioanal. Chem. 407 (2015) 7477–7485, https://doi.org/10.1007/s00216-015-8915-8.
[77] M. Tada, T. Suzuki, A. Ishii-Watabe, Development and characterization of an anti-rituximab monoclonal antibody panel, mAbs 10 (2018) 370–379, https://doi.org/10.1080/19420862.2018.1424610.
[78] D.K. Weeraratne, J. Lofgren, S. Dinnogen, S.J. Swanson, Z.D. Zhong, Development of a biosensor-based immunogenicity assay capable of blocking soluble drug target interference, J. Immunol. Methods 396 (2013) 44–55, https://doi.org/10.1016/j.jim.2013.07.010.
[79] M. Beeg, A. Nobili, B. Orsini, F. Rogai, D. Gilardi, G. Fiorino, S. Danese, M. Salmona, S. Garattini, M. Gobbi, A surface plasmon Resonance-based assay to measure serum concentrations of therapeutic antibodies and anti-drug antibodies, Sci. Rep. 9 (2019) 2064, https://doi.org/10.1038/s41598-018-37950-4.
[80] M. Beeg, C. Burti, E. Allocati, C. Ciafardini, R. Banzi, A. Nobili, F. Caprioli, S. Garattini, M. Gobbi, Surface plasmon resonance unveils important pitfalls of enzyme-linked immunoassay for the detection of anti-infliximab antibodies in patients' sera, Sci. Rep. 11 (2021) 14976, https://doi.org/10.1038/s41598-021-94431-x.
[81] C. Aniol-Nielsen, H. Toft-Hansen, M. Dahlbäck, C.H. Nielsen, H. Solberg, Calibration–free concentration analysis for quantification of anti-drug specific antibodies in polyclonal positive control antibodies and in clinical samples, J. Immunol. Methods 497 (2021) 113002, https://doi.org/10.1016/j.jim.2021.113002.
[82] J.A. Lofgren, S. Dhandapani, J.J. Pennucci, C.M. Abbott, D.T. Mytych, A. Kaliyaperumal, S.J. Swanson, M.C. Mullenix, E.L.I.S.A. Comparing,

Surface Plasmon, Resonance for assessing clinical immunogenicity of panitumumab, J. Immunol. 178 (2007) 7467–7472, https://doi.org/10.4049/jimmunol.178.11.7467.
[83] M.K. Grasmeier, S. Weber, M. Treiber, M.A. Thaler, P.B. Luppa, Surface plasmon resonance assays for the therapeutic drug monitoring of Infliximab indicate clinical relevance of anti-infliximab antibody binding properties, Clin. Chem. Lab. Med. 61 (2023) 1255–1265, https://doi.org/10.1515/cclm-2022-0949.
[84] G. Young, N. Hundt, D. Cole, A. Fineberg, J. Andrecka, A. Tyler, A. Olerinyova, A. Ansari, E.G. Marklund, M.P. Collier, S.A. Chandler, O. Tkachenko, J. Allen, M. Crispin, N. Billington, Y. Takagi, J.R. Sellers, C. Eichmann, P. Selenko, L. Frey, R. Riek, M.R. Galpin, W.B. Struwe, J.L.P. Benesch, P. Kukura, Quantitative mass imaging of single biological macromolecules, Science 360 (2018) 423–427, https://doi.org/10.1126/science.aar5839.
[85] C. Wagner, F.F. Fuchsberger, B. Innthaler, M. Lemmerer, R. Birner-Gruenberger, Quantification of empty, partially filled and full adeno-associated virus vectors using mass photometry, Indian J. Manag. Sci. 24 (2023) 11033, https://doi.org/10.3390/ijms241311033.
[86] Y. Li, W.B. Struwe, P. Kukura, Single molecule mass photometry of nucleic acids, Nucleic Acids Res. 48 (2020), https://doi.org/10.1093/nar/gkaa632 e97–e97.
[87] A. Olerinyova, A. Sonn-Segev, J. Gault, C. Eichmann, J. Schimpf, A.H. Kopf, L.S. P. Rudden, D. Ashkinadze, R. Bomba, L. Frey, J. Greenwald, M.T. Degiacomi, R. Steinhilper, J.A. Killian, T. Friedrich, R. Riek, W.B. Struwe, P. Kukura, Mass photometry of membrane proteins, Chem 7 (2021) 224–236, https://doi.org/10.1016/j.chempr.2020.11.011.
[88] D. Wu, G. Piszczek, Measuring the affinity of protein-protein interactions on a single-molecule level by mass photometry, Anal. Biochem. 592 (2020) 113575, https://doi.org/10.1016/j.ab.2020.113575.
[89] F. Soltermann, E.D.B. Foley, V. Pagnoni, M. Galpin, J.L.P. Benesch, P. Kukura, W. B. Struwe, Quantifying protein–protein interactions by molecular counting with mass photometry, Angew. Chem. Int. Ed. 59 (2020) 10774–10779, https://doi.org/10.1002/anie.202001578.
[90] M.A. Den Boer, S.-H. Lai, X. Xue, M.D. Van Kampen, B. Bleijlevens, A.J.R. Heck, Comparative analysis of antibodies and heavily glycosylated macromolecular immune complexes by size-exclusion chromatography multi-angle light scattering, native charge detection mass spectrometry, and mass photometry, Anal. Chem. 94 (2022) 892–900, https://doi.org/10.1021/acs.analchem.1c03656.
[91] V. Yin, S.-H. Lai, T.G. Caniels, P.J.M. Brouwer, M. Brinkkemper, Y. Aldon, H. Liu, M. Yuan, I.A. Wilson, R.W. Sanders, M.J. Van Gils, A.J.R. Heck, Probing affinity, avidity, anticooperativity, and competition in antibody and receptor binding to the SARS-CoV-2 spike by single particle mass analyses, ACS Cent. Sci. 7 (2021) 1863–1873, https://doi.org/10.1021/acscentsci.1c00804.
[92] T. Reinert, P. Houzé, N. Mignet, A. Naghizade, L. Alez-Martin, O. Hernandez-Alba, A. Leclerc, S. Cianferani, R. Gahoual, Y.-N. Francois, Therapeutic monoclonal antibody—antidrug antibody affinity constant determination using capillary electrophoresis–tandem mass spectrometry, Anal. Chem. 96 (2024) 19286–19293, https://doi.org/10.1021/acs.analchem.4c02932.
[93] L. Yel, C.J. Rabbat, C. Cunningham-Rundles, J.S. Orange, T.R. Torgerson, J. W. Verbsky, Y. Wang, M. Fu, T.S. Robins, M.S. Edwards, J. Nymann-Andersen, A novel targeted screening tool for hypogammaglobulinemia: measurement of Serum Immunoglobulin (IgG, IgM, IgA) levels from dried blood spots (Ig-DBS assay), J. Clin. Immunol. 35 (2015) 573–582, https://doi.org/10.1007/s10875-015-0184-y.
[94] S. Pisanu, G. Biosa, L. Carcangiu, S. Uzzau, D. Pagnozzi, Comparative evaluation of seven commercial products for human serum enrichment/depletion by shotgun proteomics, Talanta 185 (2018) 213–220, https://doi.org/10.1016/j.talanta.2018.03.086.
[95] N. Ahsan, L. Fornelli, F.Z. Najar, S. Gamagedara, M.R. Hossan, R.S.P. Rao, U. Punyamurtula, A. Bauer, Z. Yang, S.B. Foster, M.A. Kane, Proteomics evaluation of five economical commercial abundant protein depletion kits for enrichment of diseases-specific biomarkers from blood serum, Proteomics 23 (2023) 2300150, https://doi.org/10.1002/pmic.202300150.
[96] M.A.Z. Arruda, J.R. Jesus, R.M. Galazzi, Electrophoresis | gel electrophoresis: two-dimensional gel electrophoresis of proteins☆, in: P. Worsfold, C. Poole, A. Townshend, M. Miró (Eds.), Encyclopedia of Analytical Science, third ed., Academic Press, Oxford, 2019, pp. 493–504, https://doi.org/10.1016/B978-0-12-409547-2.14480-4.
[97] T.T.T.N. Nguyen, U.H. Mistarz, N. Costa, A. Herbet, D. Boquet, F. Becher, K. D. Rand, Investigating the utility of minimized sample preparation and high-resolution mass spectrometry for quantification of monoclonal antibody drugs, J. Pharmaceut. Biomed. Anal. 159 (2018) 384–392, https://doi.org/10.1016/j.jpba.2018.07.012.
[98] P. McDonald, C. Victa, J.N. Carter-Franklin, R. Fahrner, Selective antibody precipitation using polyelectrolytes: a novel approach to the purification of monoclonal antibodies, Biotechnol. Bioeng. 102 (2009) 1141–1151, https://doi.org/10.1002/bit.22127.
[99] J. Sieberz, B. Stanislawski, K. Wohlgemuth, G. Schembecker, Identification of parameter interactions influencing the precipitation of a monoclonal antibody with anionic polyelectrolytes, Separ. Purif. Technol. 127 (2014) 165–173, https://doi.org/10.1016/j.seppur.2014.02.033.
[100] A. Schäfer, P. Sagelsdorff, B. Hock, P. Bhuyan, N. Moullan, C. Siethoff, HPLC–MS/MS-based quantification of human monoclonal antibodies targeting SARS-CoV-2 in the presence of endogenous SARS-CoV-2 antibodies in human serum, Anal. Bioanal. Chem. (2024), https://doi.org/10.1007/s00216-024-05375-w.
[101] G.T. Hermanson, Chapter 15-Immobilization of ligands on chromatography supports, in: G.T. Hermanson (Ed.), Bioconjugate Techniques, third ed., Academic Press, Boston, 2013, pp. 589–740, https://doi.org/10.1016/B978-0-12-382239-0.00015-7.
[102] R. Legeron, F. Xuereb, S. Chaignepain, A.-P. Gadeau, S. Claverol, J.-W. Dupuy, S. Djabarouti, T. Couffinhal, J.-M. Schmitter, D. Breilh, A new reliable, transposable and cost-effective assay for absolute quantification of total plasmatic bevacizumab by LC–MS/MS in human plasma comparing two internal standard calibration approaches, J. Chromatogr. B 1070 (2017) 43–53, https://doi.org/10.1016/j.jchromb.2017.10.042.
[103] H.-H. Chiu, Y.-J. Tsai, C. Lo, H.-W. Liao, C.-H. Lin, S.-C. Tang, C.-H. Kuo, Development of an LC-MS/MS method to simultaneously quantify therapeutic mAbs and estimate hematocrit values in dried blood spot samples, Anal. Chim. Acta 1189 (2022) 339231, https://doi.org/10.1016/j.aca.2021.339231.
[104] H.-H. Chiu, I.-L. Tsai, Y.-S. Lu, C.-H. Lin, C.-H. Kuo, Development of an LC-MS/MS method with protein G purification strategy for quantifying bevacizumab in human plasma, Anal. Bioanal. Chem. 409 (2017) 6583–6593, https://doi.org/10.1007/s00216-017-0607-0.
[105] T.W. Mak, M.E. Saunders, 7-Exploiting antigen–antibody interaction, in: T. W. Mak, M.E. Saunders (Eds.), The Immune Response, Academic Press, Burlington, 2006, pp. 147–177, https://doi.org/10.1016/B978-012088451-3.50009-0.
[106] J.-F. Jourdil, D. Lebert, E. Gautier-Veyret, F. Lemaitre, B. Bonaz, G. Picard, J. Tonini, F. Stanke-Labesque, Infliximab quantitation in human plasma by liquid chromatography-tandem mass spectrometry: towards a standardization of the methods? Anal. Bioanal. Chem. 409 (2017) 1195–1205, https://doi.org/10.1007/s00216-016-0045-4.
[107] M. El Amrani, M.P.H. Van Den Broek, C. Göbel, E.M. Van Maarseveen, Quantification of active Infliximab in human serum with liquid chromatography–tandem mass spectrometry using a tumor necrosis factor alpha -based pre-analytical sample purification and a stable isotopic labeled Infliximab bio-similar as internal standard: a target-based, sensitive and cost-effective method, J. Chromatogr. A 1454 (2016) 42–48, https://doi.org/10.1016/j.chroma.2016.05.070.
[108] T. Reinert, P. Houzé, Y.-N. Francois, R. Gahoual, Enhancing affinity purification of monoclonal antibodies from human serum for subsequent CZE-MS analysis, J. Chromatogr. B 1234 (2024) 123974, https://doi.org/10.1016/j.jchromb.2023.123974.
[109] B. Sun, J. Liu, P. Cai, J. Wu, W. Liu, H. Hu, L. Liu, Aptamer-based sample purification for mass spectrometric quantification of trastuzumab in human serum, Talanta 257 (2023) 124349, https://doi.org/10.1016/j.talanta.2023.124349.
[110] M. Fresnais, R. Longuespée, M. Sauter, T. Schaller, M. Arndt, J. Krauss, A. Blank, W.E. Haefeli, J. Burhenne, Development and validation of an LC–MS-Based quantification assay for new therapeutic antibodies: application to a novel therapy against Herpes simplex virus, ACS Omega 5 (2020) 24329–24339, https://doi.org/10.1021/acsomega.0c02547.
[111] A. Millet, R. Pescarmona, A. Belot, C. Machon, Y. Jamilloux, J. Guitton, Quantification of canakinumab in human plasma by liquid chromatography-high resolution mass spectrometry, J. Chromatogr. B 1211 (2022) 123475, https://doi.org/10.1016/j.jchromb.2022.123475.
[112] V.P. Gaspar, S. Ibrahim, C.A. Sobsey, V.R. Richard, A. Spatz, R.P. Zahedi, C. H. Borchers, Direct and precise measurement of bevacizumab levels in human plasma based on controlled methionine oxidation and multiple reaction monitoring, ACS Pharmacol. Transl. Sci. 3 (2020) 1304–1309, https://doi.org/10.1021/acsptsci.0c00134.
[113] R. Russo, C. Rega, A. Caporale, G. Tonon, S. Scaramuzza, F. Selis, M. Ruvo, A. Chambery, Ultra-performance liquid chromatography/multiple reaction monitoring mass spectrometry quantification of trastuzumab in human serum by selective monitoring of a specific peptide marker from the antibody complementarity-determining regions, Rapid Commun. Mass Spectrom. 31 (2017) 1184–1192, https://doi.org/10.1002/rcm.7898.
[114] X. Zhong, S. Nayak, L. Guo, S. Raidas, Y. Zhao, R. Weiss, M. Andisik, C. Elango, G. Sumner, S.C. Irvin, M.A. Partridge, H. Yan, S.Y. E, H. Qiu, Y. Mao, A. Torri, N. Li, Liquid chromatography-multiple reaction monitoring-mass spectrometry assay for quantitative measurement of therapeutic antibody cocktail REGEN-COV concentrations in COVID-19 patient serum, Anal. Chem. 93 (2021) 12889–12898, https://doi.org/10.1021/acs.analchem.1c01613.
[115] E.I. Hallin, T.T. Serkland, T.K. Bjånes, S. Skrede, High-throughput, low-cost quantification of 11 therapeutic antibodies using caprylic acid precipitation and LC-MS/MS, Anal. Chim. Acta 1313 (2024) 342789, https://doi.org/10.1016/j.aca.2024.342789.
[116] P. Matlak, H. Brozmanova, P. Sistik, I. Kacirova, P. Hradilek, M. Grundmann, Liquid chromatography - tandem mass spectrometry method for determination of natalizumab in serum and cerebrospinal fluid of patients with multiple sclerosis, J. Pharmaceut. Biomed. Anal. 234 (2023) 115542, https://doi.org/10.1016/j.jpba.2023.115542.
[117] R. Mu, Y. Huang, J. Bouquet, J. Yuan, R.J. Kubiak, E. Ma, S. Naser, W.R. Mylott, O.A. Ismaiel, A.M. Wheeler, R. Burkart, D.F. Cortes, J. Bruton, R.H. Arends, M. Liang, A.I. Rosenbaum, Multiplex hybrid antigen-capture LC-MRM quantification in sera and nasal lining fluid of AZD7442, a SARS-CoV-2-Targeting antibody combination, Anal. Chem. 94 (2022) 14835–14845, https://doi.org/10.1021/acs.analchem.2c01320.
[118] M.E. Amrani, L. Gerencser, A.D.R. Huitema, C.E. Hack, M. Van Luin, K.C.M. Van Der Elst, A generic sample preparation method for the multiplex analysis of seven therapeutic monoclonal antibodies in human plasma or serum with liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1655 (2021) 462489, https://doi.org/10.1016/j.chroma.2021.462489.

[119] S. Schokker, F. Fusetti, F. Bonardi, R.J. Molenaar, R.A.A. Mathôt, H.W.M. Van Laarhoven, Development and validation of an LC-MS/MS method for simultaneous quantification of co-administered trastuzumab and pertuzumab, mAbs 12 (2020) 1795492, https://doi.org/10.1080/19420862.2020.1795492.
[120] J.-F. Jourdil, B. Némoz, E. Gautier-Veyret, C. Romero, F. Stanke-Labesque, Simultaneous quantification of adalimumab and infliximab in human plasma by liquid chromatography–tandem mass spectrometry, Ther. Drug Monit. 40 (2018) 417–424, https://doi.org/10.1097/FTD.0000000000000514.
[121] B. Nemoz, D. Ternant, S. Bailly, E. Gautier-Veyret, J. Jourdil, B. Bonaz, F. Stanke-Labesque, New steps in Infliximab therapeutic drug monitoring in patients with inflammatory bowel diseases, Br. J. Clin. Pharmacol. 85 (2019) 722–728, https://doi.org/10.1111/bcp.13845.
[122] T. Willeman, J.-F. Jourdil, E. Gautier-Veyret, B. Bonaz, F. Stanke-Labesque, A multiplex liquid chromatography tandem mass spectrometry method for the quantification of seven therapeutic monoclonal antibodies: application for adalimumab therapeutic drug monitoring in patients with Crohn's disease, Anal. Chim. Acta 1067 (2019) 63–70, https://doi.org/10.1016/j.aca.2019.03.033.
[123] C. Marin, N. Khoudour, A. Millet, D. Lebert, P. Bros, F. Thomas, D. Ternant, B. Lacarelle, J. Guitton, J. Ciccolini, B. Blanchet, Cross-validation of a multiplex LC-MS/MS method for assaying mAbs plasma levels in patients with cancer: a GPCO-UNICANCER study, Pharmaceuticals 14 (2021) 796, https://doi.org/10.3390/ph14080796.
[124] A. Hentschel, G. Piontek, R. Dahlmann, P. Findeisen, R. Sakson, P. Carbow, T. Renné, Y. Reinders, A. Sickmann, Highly sensitive therapeutic drug monitoring of infliximab in serum by targeted mass spectrometry in comparison to ELISA data, Clin. Proteom. 21 (2024) 16, https://doi.org/10.1186/s12014-024-09464-x.
[125] E. Güven, K. Duus, M.C. Lydolph, C.S. Jørgensen, I. Laursen, G. Houen, Non-specific binding in solid phase immunoassays for autoantibodies correlates with inflammation markers, J. Immunol. Methods 403 (2014) 26–36, https://doi.org/10.1016/j.jim.2013.11.014.
[126] M.D. Krasowski, M.G. Siam, M. Iyer, S. Ekins, Molecular similarity methods for predicting cross-reactivity with therapeutic drug monitoring immunoassays, Ther. Drug Monit. 31 (2009) 337–344, https://doi.org/10.1097/FTD.0b013e31819c1b83.
[127] Y. Xiao, S.N. Isaacs, Enzyme-linked immunosorbent assay (ELISA) and blocking with bovine serum albumin (BSA)—Not all BSAs are alike, J. Immunol. Methods 384 (2012) 148–151, https://doi.org/10.1016/j.jim.2012.06.009.
[128] M.A.V. Willrich, D.L. Murray, D.R. Barnidge, P.M. Ladwig, M.R. Snyder, Quantitation of infliximab using clonotypic peptides and selective reaction monitoring by LC–MS/MS, Int. Immunopharmacol. 28 (2015) 513–520, https://doi.org/10.1016/j.intimp.2015.07.007.
[129] T. Reinert, R. Gahoual, N. Mignet, A. Kulus, M. Allez, P. Houzé, Y.-N. François, Simultaneous quantification and structural characterization of monoclonal antibodies after administration using capillary zone electrophoresis-tandem mass spectrometry, J. Pharmaceut. Biomed. Anal. 233 (2023) 115446, https://doi.org/10.1016/j.jpba.2023.115446.
[130] M.E. Amrani, K.C.M. Van Der Elst, A.D.R. Huitema, M. Van Luin, Middle-up quantification of therapeutic monoclonal antibodies in human plasma with two dimensional liquid chromatography high resolution mass spectrometry: Adalimumab as a proof of principle, J. Chromatogr. A 1665 (2022) 462840, https://doi.org/10.1016/j.chroma.2022.462840.
[131] S. Liu, J. Yang, S. Yang, Q. Tan, L. Dai, G. Fan, D. Wu, Z. Zhang, J. Yao, Y. Shi, X. Han, Development and validation of a UPLC-MS/MS method to quantitate anti-PD1 monoclonal antibody (Toripalimab), and comparison with electrochemiluminescence immunoassay, J. Pharmaceut. Biomed. Anal. 209 (2022) 114515, https://doi.org/10.1016/j.jpba.2021.114515.
[132] J.R. Mills, D. Cornec, S. Dasari, P.M. Ladwig, A.M. Hummel, M. Cheu, D. L. Murray, M.A. Willrich, M.R. Snyder, G.S. Hoffman, C.G.M. Kallenberg, C. A. Langford, P.A. Merkel, P.A. Monach, P. Seo, R.F. Spiera, E.W. St Clair, J. H. Stone, U. Specks, D.R. Barnidge, Using mass spectrometry to quantify rituximab and perform individualized immunoglobulin phenotyping in ANCA-associated vasculitis, Anal. Chem. 88 (2016) 6317–6325, https://doi.org/10.1021/acs.analchem.6b00544.
[133] F. Becher, J. Ciccolini, D.-C. Imbs, C. Marin, C. Fournel, C. Dupuis, N. Fakhry, B. Pourroy, A. Ghettas, A. Pruvost, C. Junot, F. Duffaud, B. Lacarelle, S. Salas, A simple and rapid LC-MS/MS method for therapeutic drug monitoring of cetuximab: a GPCO-UNICANCER proof of concept study in head-and-neck cancer patients, Sci. Rep. 7 (2017) 2714, https://doi.org/10.1038/s41598-017-02821-x.
[134] P.M. Ladwig, D.R. Barnidge, M.A.V. Willrich, Quantification of the IgG2/4 kappa monoclonal therapeutic eculizumab from serum using isotype specific affinity purification and microflow LC-ESI-Q-TOF mass spectrometry, J. Am. Soc. Mass Spectrom. 28 (2017) 811–817, https://doi.org/10.1007/s13361-016-1566-y.
[135] N. Scheffe, R. Schreiner, A. Thomann, P. Findeisen, Development of a mass spectrometry-based method for quantification of ustekinumab in serum specimens, Ther. Drug Monit. 42 (2020) 572–577, https://doi.org/10.1097/FTD.0000000000000734.
[136] M. El Amrani, C.L. Szanto, C.E. Hack, A.D.R. Huitema, S. Nierkens, E.M. Van Maarseveen, Quantification of total dinutuximab concentrations in neuroblastoma patients with liquid chromatography tandem mass spectrometry, Anal. Bioanal. Chem. 410 (2018) 5849–5858, https://doi.org/10.1007/s00216-018-1198-0.
[137] H.-H. Chiu, Y.-J. Tsai, C. Lo, C.-H. Lin, I.-L. Tsai, C.-H. Kuo, Development of an efficient mAb quantification assay by LC-MS/MS using rapid on-bead digestion, Anal. Chim. Acta 1193 (2022) 339319, https://doi.org/10.1016/j.aca.2021.339319.
[138] M. El Amrani, S.M. Bosman, A.C. Egas, C.E. Hack, A.D.R. Huitema, E.M. Van Maarseveen, Simultaneous quantification of free adalimumab and Infliximab in human plasma using a target-based sample purification and liquid chromatography–tandem mass spectrometry, Ther. Drug Monit. 41 (2019) 640–647, https://doi.org/10.1097/FTD.0000000000000633.
[139] L. Liu, B. Sun, J. Cai, J. Wang, W. Liu, H. Hu, S. Chen, J. Wu, Simultaneous quantification of co-administered trastuzumab and pertuzumab in serum based on nano-surface and molecular-orientation limited (nSMOL) proteolysis, RSC Adv. 14 (2024) 19550–19559, https://doi.org/10.1039/D4RA03060E.
[140] M. El Amrani, C. Göbel, A.C. Egas, S. Nierkens, C.E. Hack, A.D.R. Huitema, E. M. Van Maarseveen, Quantification of neutralizing anti-drug antibodies and their neutralizing capacity using competitive displacement and tandem mass spectrometry: infliximab as proof of principle, J. Translation. Autoimmun. 1 (2019) 100004, https://doi.org/10.1016/j.jtauto.2019.100004.
[141] L.-Z. Chen, D. Roos, E. Philip, Development of Immunocapture-LC/MS assay for simultaneous ADA isotyping and semiquantitation, J. Immunol. Res. 2016 (2016) 1–14, https://doi.org/10.1155/2016/7682472.
[142] E.H. Smeijsters, K.C.M. Van Der Elst, A. Visch, C. Göbel, F.C. Loeff, T. Rispens, A. D.R. Huitema, M. Van Luin, M. El Amrani, Optimization of a quantitative anti-drug antibodies against Infliximab assay with the liquid chromatography-tandem mass spectrometry: a method validation study and future perspectives, Pharmaceutics 15 (2023) 1477, https://doi.org/10.3390/pharmaceutics15051477.
[143] N. Iwamoto, A. Hamada, T. Shimada, Antibody drug quantitation in coexistence with anti-drug antibodies on nSMOL bioanalysis, Anal. Biochem. 540–541 (2018) 30–37, https://doi.org/10.1016/j.ab.2017.11.002.
[144] T. Rispens, A.V. Leeuwen, A. Vennegoor, J. Killestein, R.C. Aalberse, G. J. Wolbink, L.A. Aarden, Measurement of serum levels of natalizumab, an immunoglobulin G4 therapeutic monoclonal antibody, Anal. Biochem. 411 (2011) 271–276, https://doi.org/10.1016/j.ab.2011.01.001.
[145] L. He, L.C. Anderson, D.R. Barnidge, D.L. Murray, C.L. Hendrickson, A. G. Marshall, Analysis of monoclonal antibodies in human serum as a model for clinical monoclonal gammopathy by use of 21 tesla FT-ICR top-down and middle-down MS/MS, J. Am. Soc. Mass Spectrom. 28 (2017) 827–838, https://doi.org/10.1007/s13361-017-1602-6.
[146] L. Zhang, L.A. Vasicek, S. Hsieh, S. Zhang, K.P. Bateman, J. Henion, Top-down Lc–Ms quantitation of intact denatured and native monoclonal antibodies in biological samples, Bioanalysis 10 (2018) 1039–1054, https://doi.org/10.4155/bio-2017-0282.
[147] P. Legrand, S. Dufaÿ, N. Mignet, P. Houzé, R. Gahoual, Modeling study of long-term stability of the monoclonal antibody infliximab and biosimilars using liquid-chromatography–tandem mass spectrometry and size-exclusion chromatography–multi-angle light scattering, Anal. Bioanal. Chem. 415 (2023) 179–192, https://doi.org/10.1007/s00216-022-04396-7.
[148] D. Sarin, S. Kumar, A.S. Rathore, Monitoring oxidation in recombinant monoclonal antibodies at subunit level through two-dimensional liquid chromatography coupled with mass spectrometry, J. Chromatograph. Open 3 (2023) 100086, https://doi.org/10.1016/j.jcoa.2023.100086.
[149] J.C. Tran, D. Tran, A. Hilderbrand, N. Andersen, T. Huang, K. Reif, I. Hotzel, E. G. Stefanich, Y. Liu, J. Wang, Automated affinity capture and On-Tip digestion to accurately quantitate *in Vivo* deamidation of therapeutic antibodies, Anal. Chem. 88 (2016) 11521–11526, https://doi.org/10.1021/acs.analchem.6b02766.
[150] P. Bults, R. Bischoff, H. Bakker, J.A. Gietema, N.C. Van De Merbel, LC-MS/MS-Based monitoring of *in vivo* protein biotransformation: quantitative determination of trastuzumab and its deamidation products in human plasma, Anal. Chem. 88 (2016) 1871–1877, https://doi.org/10.1021/acs.analchem.5b04276.
[151] L. Lu, X. Liu, C. Zuo, J. Zhou, C. Zhu, Z. Zhang, M. Fillet, J. Crommen, Z. Jiang, Q. Wang, In vitro/in vivo degradation analysis of trastuzumab by combining specific capture on HER2 mimotope peptide modified material and LC-QTOF-MS, Anal. Chim. Acta 1225 (2022) 340199, https://doi.org/10.1016/j.aca.2022.340199.
[152] R. Gahoual, A. Beck, E. Leize-Wagner, Y.-N. François, Cutting-edge capillary electrophoresis characterization of monoclonal antibodies and related products, J. Chromatogr. B 1032 (2016) 61–78, https://doi.org/10.1016/j.jchromb.2016.05.028.
[153] R. Gahoual, J.-M. Busnel, A. Beck, Y.-N. François, E. Leize-Wagner, Full Antibody Primary Structure, Microvariant, Characterization in a single injection using transient isotachophoresis and sheathless capillary electrophoresis–tandem mass spectrometry, Anal. Chem. 86 (2014) 9074–9081, https://doi.org/10.1021/ac502378e.
[154] J. Giorgetti, A. Lechner, E. Del Nero, A. Beck, Y.-N. François, E. Leize-Wagner, Intact monoclonal antibodies separation and analysis by sheathless capillary electrophoresis-mass spectrometry, Eur. J. Mass Spectrom. 25 (2019) 324–332, https://doi.org/10.1177/1469066718807798.
[155] K. Jooß, J. Hühner, S. Kiessig, B. Moritz, C. Neusüß, Two-dimensional capillary zone electrophoresis–mass spectrometry for the characterization of intact monoclonal antibody charge variants, including deamidation products, Anal. Bioanal. Chem. 409 (2017) 6057–6067, https://doi.org/10.1007/s00216-017-0542-0.
[156] T. Reinert, P. Houzé, N. Mignet, Y.-N. François, R. Gahoual, Post-translational modifications comparative identification and kinetic study of infliximab innovator and biosimilars in serum using capillary electrophoresis-tandem mass spectrometry, J. Pharmaceut. Biomed. Anal. 234 (2023) 115541, https://doi.org/10.1016/j.jpba.2023.115541.