Journal of Chromatography A 1655 (2021) 462489

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

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

Mohsin El Amrani[a,*], Laszlo Gerencser[a], Alwin D.R. Huitema[a,b,c], C. Erik Hack[d], Matthijs van Luin[a], Kim C.M. van der Elst[a]

[a] Department of Clinical Pharmacy, Division Laboratories, Pharmacy and Biomedical Genetics, University Medical Centre Utrecht, Utrecht, the Netherlands
[b] Department of Pharmacy & Pharmacology, Netherlands Cancer Institute, Amsterdam, the Netherlands
[c] Department of Pharmacology, Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands
[d] Center of Translational Immunology, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

ARTICLE INFO

Article history:
Received 14 June 2021
Revised 3 August 2021
Accepted 18 August 2021
Available online 27 August 2021

Keywords:
Ammonium sulfate
Multiplex analysis
Therapeutic monoclonal antibody
Quantification
Liquid chromatography-tandem mass spectrometry
LC-MS/MS

ABSTRACT

Due to the increasing number of therapeutic monoclonal antibodies (mAbs) used in the clinic, there is an increasing need for robust analytical methods to quantify total mAb concentrations in human plasma for clinical studies and therapeutic drug monitoring. We developed an easy, rapid, and robust sample preparation method for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. The method was validated for infliximab (IFX), rituximab (RTX), cetuximab (CTX), dupilumab (DPL), dinutuximab (DNX), vedolizumab (VDZ), and emicizumab (EMZ). Saturated ammonium sulfate (AS) was used to precipitate immunoglobulins in human plasma. After centrifugation, supernatant containing albumin was decanted, and the precipitated immunoglobulin fraction was re-dissolved in buffer containing 6M guanidine. This fraction was then completely denatured, reduced, alkylated, and trypsin digested. Finally, signature peptides from the seven mAbs were simultaneously quantified on LC-MS/MS together with their internal standards stable isotopically labeled peptide counterparts. The linear dynamic ranges (1 – 512 mg/L) of IFX, CTX, RTX, and EMZ showed excellent ($R2 > 0.999$) linearity and those of DPL, DNX, and VDZ showed good ($R2 > 0.995$) linearity. The method was validated in accordance with the EMA guidelines. EDTA plasma, sodium citrate plasma, heparin plasma, and serum yielded similar results. Prepared samples were stable at room temperature (20°C) and at 5°C for 3 days, and showed no decline in concentration for all tested mAbs. This described method, which has the advantage of an easy, rapid, and robust pre-analytical sample preparation, can be used as a template to quantify other mAbs in human plasma or serum.

1. Introduction

Approximately 40% of newly FDA-approved drugs in the last three years were therapeutic monoclonal antibodies (mAbs). This percentage has steadily increased in the past decades and probably this trend will continue in the nearby future [1-3]. The investigation of the pharmacokinetics of mAbs during preclinical studies and clinical trials is an essential part of drug development and approval [4, 5]. Post approval, phase 4 trials are conducted to study the drug in clinical practice and to study the long-term risks and benefits. To study pharmacokinetic properties and drug exposure, bioanalytical methods are required for the quantification of mAbs in biological matrices. This is especially important since these large biomolecules are capable of inducing an immune response in patients, triggering the generation of anti-drug antibodies (ADA) which can ultimately lead to treatment failure due to low drug exposure.

Traditionally, ligand binding assays, such as ELISA, which rely on immunoaffinity interaction with antibodies, have been used for this purpose. However, the development of these antibodies, with high avidity and specificity towards the therapeutic protein, can

* Corresponding author at: University Medical Center Utrecht, Heidelberglaan 100, Room nr D.00.318A, Internal post nr. D.00.204, P.O. Box 85500, 3508 GA Utrecht, the Netherlands.
E-mail address: m.elamrani@umcutrecht.nl (M.E. Amrani).

https://doi.org/10.1016/j.chroma.2021.462489

be time consuming [6-9]. In addition, ELISA methods can suffer from aspecific binding of patient IgG to the solid-phase, increasing background and decreasing sensitivity. Quantifying mAbs with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is an attractive alternative for the conventional ELISA approach, since it does not have these limitations [10-13]. Furthermore, LC-MS/MS possesses notable advantages over several other technologies, such as a faster method development, multiplexing ability, a wider linear dynamic range, and higher selectivity [14-17]. However, it can be challenging to select an appropriate and feasible sample preparation method for quantification of the target mAb with LC-MS/MS. Targeted sample preparation, using immunoaffinity enrichment to pull down the therapeutic mAb with a fixed anti-idiotypic- antibody or ligand, can attain a higher sensitivity but, like ELISA, relies on specific ligands and antibodies [15, 18-21]. Furthermore, while these methods measure the free therapeutic antibody fraction, quantification of the total therapeutic antibody, next to the free fraction, could provide insight into the levels of anti-drug antibody interactions and/or ligand-antibody interactions [6].

There are numerous sample preparation methods available to measure the total antibody fraction. Preparation methods on the basis of FC-pull down, using Protein A/G or anti-FC antibodies, have been used in the past and showed low detection limits in controlled animal studies [22-28]. Unfortunately, the sensitivity in patients may vary depending on the level of endogenous immunoglobulins (IgG), possibly due to competition between endogenous IgG and the therapeutic antibody for the binding sites [11]. Pellet digestion with methanol precipitation has also found widespread use, since it does not suffer from the capacity issues that are mentioned above [9, 29-35]. However, methanol does not remove albumin efficiently [36, 37]. Also, after centrifugation, the pellet is hard to re-dissolve in buffer solution for subsequent reduction and alkylation, which leads to suboptimal reaction conditions. One study shows that ammonium sulfate (AS) may assure efficient albumin removal without denaturing the mAb fraction [38]. This would allow for faster dissolution of the pellet in buffer which is ideal for thorough reduction and alkylation which ultimately would facilitate efficient digestion of the immunoglobulin fraction.

The purpose of this study was to develop and to validate an LC-MS/MS method for the simultaneous quantification of infliximab (IFX), rituximab (RTX), cetuximab (CTX), dupilumab (DPL), dinutuximab (DNX), vedolizumab (VDZ), and emicizumab (EMZ) in human plasma to support Therapeutic Drug Monitoring (TDM) and phase 4 clinical studies and to show the universal applicability of this generic sample preparation method for LC-MS/MS determination of mAbs.

2. Materials and methods

2.1. Chemicals and reagents

IFX (Remicade®) 10 mg/mL was obtained from Johnson and Johnson (NJ, United States), RTX (MabThera®) 10 mg/mL and EMZ (Hemlibra®) 150 mg/mL from Roche (Bazel, Zwitserland), CTX (Erbitux®) 5 mg/mL from Merck (Darmstadt, Germany), DPL (Dupixent®) 150 mg/mL from Senofi Genzyme (MA, United States), DNX (Isquette®) 4.5 mg/mL from Rentschler Biotechnologie GmbH (Laupheim, Germany), VDZ (Entyvio®) 60 mg/mL from Takeda (Tokio, Japan). Internal standard (IS) stable isotopically labeled (SIL) peptides (*) (RTX; ASGYTFTSYNMHWVK*), (CTX; YASESISGIPSR*), (DPL; LSITIRPR*), (DTX; SSSTAYMHLK*), (VDZ; LEWIGEIDPSESNTNYNQK*), and (EMZ; SGGSIYNEEFQDR*) were obtained from Pepscan Presto BV (Lelystad, The Netherlands). IFX containing SIL arginine and lysine was obtained from promise advanced proteomics (Grenoble, France). TPCK-Trypsin was supplied by Thermo Scientific as a lyophilized powder and was dissolved in acetic acid (50 mM) to a concentration of 10 µg/µL, aliquoted in Eppendorf LoBind Microcentrifuge tubes and stored at -80°C. All other chemicals, reagents, and LC-MS grade mobile phase solvents were obtained from Sigma-Aldrich (Saint Louis, MO).

2.2. Preparation of standards, internal standard and QCs

A mixed working standard solution of IFX 128 mg/L, CTX 256 mg/L, RTX 512 mg/L, DNX 128 mg/L, DPL 256 mg/L, VDZ 128 mg/L, and EMZ 512 mg/L was prepared in pulled human plasma from healthy volunteers. This was then serially diluted with human plasma to obtain the following calibration standards for IFX, DNX, and VDZ (128 – 64 – 32 – 16 – 8 – 4 – 2 – 1 mg/L), for CTX and DPL (256 – 128 – 64 – 32 – 16 – 8 – 4 – 2 mg/L), and for RTX and EMZ (512 - 256 – 128 – 64 – 32 – 16 – 8 – 4 mg/L).

SIL peptide IS were diluted to 0.5 mg/L and SIL IFX IS to 10 mg/L in TRIS buffer pH 8.5, 50 mM containing 0.5% octyl glucoside (OG). Quality Control samples (QCs) were prepared in pooled plasma from a separate batch, at the lower limit of quantification (LLOQ) of 1 mg/L for IFX, DNX and VDZ, 2 mg/L for CTX and DPL, and 4 mg/L for RTX and EMZ. QC Low was 2.5 mg/L for IFX, DNX, and VDZ, 5 mg/L for CTX and DPL, and 10 mg/L for RTX and EMZ. QC Med was 50 mg/L for IFX, DNX, and VDZ, 100 mg/L for CTX and DPL, and 200 mg/L for RTX and EMZ. QC High was 100 mg/L for IFX, DNX, and VDZ, 200 mg/L for CTX and DPL, and 400 mg/L for RTX and EMZ. Aliquots of calibration standards and QC's were stored at -80°C in Lobind® Eppendorf tubes.

2.3. Instrumentation and chromatographic conditions

Sample reduction and digestion were performed on an Eppendorf® ThermoMixer C. All experiments were performed on an Vanquish UHPLC coupled to a TSQ Altis (Thermo Fisher, Waltham, MA, USA). The analytical column was Acclaim®, RSLC 120, C18, 2.1 × 100 mm, 2.2 μm particle size obtained from Thermo Fisher and was maintained at 50°C. The mobile phases were: (A) 0.1% formic acid in water; and (B) 0.1% formic acid in acetonitrile. The LC gradients in minutes per percentage of mobile phase B were 0.0 (min)/5 (%B), 9.0/35, 9.1/100, 11.0/100, 11.1/5, and 13/5. The flow rate was 0.5 ml/min and the running time was 10.5 min. The MS was operated in positive mode with spray voltage of 3.0 kV, ion transfer tube temperature 350°C, vaporizer temperature 300°C, aux gas pressure 15 Arb, sheath gas pressure 50 Arb, and collision gas pressure 2.5 mTorr. The precursor ions, product ions, Radio frequency (RF) lens, and collision energy settings are listed in Table 1.

2.4. Method development

The amino acid sequences of the therapeutic mAbs were from the Immunogenetics Information system® (http://www.imgt.org/). The variable light and heavy chains were digested in silico using Skyline® software from the University of Washington. Sequences of stable peptides with a length of 6 to 20 amino acids were compared with human genomic sequences using the basic local alignment search tool for proteins (Blastp®) (https://blast.ncbi.nlm.nih.gov). Peptides with sequences that did not match with any known human sequence were selected as potential signature peptide candidates and were optimized for LC separation. SRM transition, collision energy, and RF lens settings were optimized using Skyline®. Peptides chosen as quantifiers were required to be between 6 to 20 amino acids long, to allow for sufficient chromatographic retention, should have a high signal to noise ratio (S/N) to allow for sensitive detection and should contain stable amino acids, free of cysteine (C) or asparagine followed by glycine (NG). Finally, the signature peptides should not contain double arginine (RR) and double

Table 1
TSQ Altis SRM transitions and settings for the signature tryptic peptides quantifiers, qualifiers and their SIL internal standard (IS).

Component	Type	Precursor	Precursor [m/z]	Product [m/z]	Product fragment	CE [a]	RF [b]
DTX	Quantifier	SSSTAYMHLK	375.518	475.742	Y8	10.6	53
	IS	SSSTAYMHLK*	378.1897	479.7489	Y8	10.6	53
	Qualifier	SLEWIGAIDPYYGGTSYNQK	1131.54	1277.58	Y11	31.7	190
IFX	Quantifier	SINSATHYAESVK	469.568	603.791	Y11	12.4	56
	IS	SINSATHYAESVK*	472.24	607.798	Y11	12.4	56
	Qualifier	YASESMSGIPSR	642.798	1050.488	Y10	22.2	93
	IS	YASESMSGIPSR*	647.802	1060.4967	Y10	22.2	93
CTX	Quantifier	YASESISGIPSR	633.8199	359.2037	Y3	17.8	84
	IS	YASESISGIPSR*	638.8240	369.2120	Y3	17.8	84
	Qualifier	ASQSIGTNIHWYQQR	596.964	651.8205	Y10	14.1	94
EMZ	Quantifier	SGGSIYNEEFQDR	751.331	1100.464	Y8	23.8	110
	IS	SGGSIYNEEFQDR*	756.3355	1110.4726	Y8	23.8	110
	Qualifier	QAPGQGLEWMGDINTR	886.9229	787.3750	Y14	26.4	124
DPL	Quantifier	LSITIRPR	319.206	421.76	Y7	12	51
	IS	LSITIRPR*	322.54	426.77	Y7	12	51
	Qualifier	DYAMTWVR	521.245	763.392	Y6	15	64
VDZ	Quantifier	LEWIGEIDPSESNTNYNQK	1119.022	1281.5706	Y11	34.3	195
	IS	LEWIGEIDPSESNTNYNQK*	1123.0286	1289.5848	Y11	34.3	195
	Qualifier	LEWIGEIDPSESNTNYNQK	1119.022	429.2132	B3	37	195
	IS	LEWIGEIDPSESNTNYNQK*	1123.0286	429.2132	B3	37	195
RTX	Quantifier	ASGYTFTSYNMHWVK	597.944	817.377	Y13	15	69
	IS	ASGYTFTSYNMHWVK*	600.615	821.384	Y13	15	69
	Qualifier	GLEWIGAIYPGNGDTSYNQK	1092.0238	1180.523	Y11	31.4	185

[a] CE: Collision energy
[b] RF: Radio frequency lens
K*: Lys (13C6,15N2)
R*: Arg (13C6,15N4)
IFX= Infliximab, CTX = Cetuximab, RTX = Rituximab, DNX = Dinutuximab, DPL = Dupilumab, VDZ = Vedolizumab and EMZ = Emicizumab

lysine (KK), which are sensitive to miss cleavages. Peptides with lower S/N were chosen as qualifiers. They are used to verify that the analyte is indeed present in the sample, since there is a smaller chance of matrix interfering in two different SRM transitions from two different peptides. An in-house developed sample preparation method [39] was chosen as a template method and conditions were re-evaluated and further optimized. Sampling tubes, AS precipitation concentration, denaturation and trypsin digestion were optimized to allow for the quantification of other therapeutic mAbs. The methanol precipitation step, showed a high recovery between a wide methanol concentration range 50-80% [39] and was therefore not re-evaluated in this study. The method also utilizes SIL IFX as IS which has a double purpose in this method. First, it was used to correct for variation for IFX samples and calibration standards during sample preparation and second, to check for possible IgG pellet loss which could occur during decanting of the supernatant layer in both the AS and MeOH precipitation steps. The remaining IS solutions were chosen as SIL peptides since these were easy to synthesis and have low costs.

2.5. Sample preparation for LC-MS/MS analysis

In a Lobind® 1 mL 96-well plate, 10 µL (sample, standard, or QC) and 10 µL SIL IFX (10 mg/L) was added following 80 µL TRIS (50mM, pH 8, 0.5% OG). Then, 100 µL AS (saturated) was added to each sample followed by 1 minute mixing on a ThermoMixer at 1350 RPM. The 96 well plate was centrifuged at 4000 G for 5 minutes. The supernatant containing albumin was decanted and the pellet containing IgG was redissolved in 50 µL of TRIS buffer (100 mM, pH 8.5) containing 6M guanidine and 20 mM DTT. Then, the plate was placed in a ThermoMixer at 60°C and 1000 RPM for 30 minutes to dissolve, reduce, and denature the IgG. Samples were alkylated by adding 20 µL iodoacetamide (IAA) (100 mM dissolved in ultrapure water) and placed on a ThermoMixer at 37°C for 30 minutes in the dark. Then, 150 µL ultrapure water was added and mixed for 1 minute to dissolve and ultimately wash away the guanidine and IAA that otherwise would interfere with tryptic digestion. After mixing, methanol was added to precipitate the IgG fraction once again and the well plate was centrifuged at 4000 G for 5 minutes. The supernatant containing guanidine and IAA was decanted. Then, 90 µL mixed IS SIL peptide solution (0.5 mg/L) was added, followed by 10 µL Trypsin (2 µg/µL) and the samples were placed on the ThermoMixer for overnight digestion (15h) at 37°C and 1000 RPM. Trypsin activity was stopped by adding 20 µL 10% formic acid in acetonitrile. Finally, 10 µL was injected on the LC-MS/MS.

2.6. Ammonium sulfate precipitation

The effect of AS concentration, within the range 35.5-54.5%, was examined in relation to the signature peptides signal intensity. QC high was diluted 10 times in human plasma, thereafter 10 µL was pipetted 15 times in a 1 mL LoBind® deep well plate. Then, 90µL Tris (pH 8, 0.5% OG) was added and mixed, followed by the addition of 55 (35.5%), 65 (39.4%), 80 (44.4%), 100 (50%), and 120 µL (54.5%) saturated AS in triplicates. Samples were subsequently prepared and analyzed following the procedure described above. Relative recovery was calculated for each component at various AS concentrations, by dividing the signal obtained at a specific concentration by the highest signal, then multiplying by 100%. This allowed for all components to be visibly plotted in one graph.

2.7. Sample preparation

The widely used methanol (MeOH) pellet digestion method was compared to AS and guanidine sample preparation to show the added value of presented method. The reference method used for comparison was from Hao Jiang et al [29] and was modified slightly to make a fair comparison possible. A mixed standard containing 100 mg/L of each therapeutic mAb was processed in fivefold according to the sample preparation described above and according to the MeOH pellet digestion described by Hao Jiang et al.

The MeOH pellet digestion principle was as followed. 10 µL mixed standard was pipetted in fivefold in 1.5 mL Lobind® Eppendorf tubes. A pellet was achieved by adding 40 µl MeOH and vortexing at 2000 rpm for 2 minutes. Samples were centrifuged at 200g for 2 minutes and the supernatant was removed by careful pipetting. Then, 90 µL mixed IS SIL peptide solution (0.5 mg/L) was added and mixed at 2000 rpm for 2 minutes. Samples were reduced by adding 5 µL 100mM DTT at 60°C for 60 minutes and alkylated with 10 µL IAA 100 mM at 30°C for 30 minutes both at 1000 rpm. Finally, 10 µL Trypsin (2 µg/µL) was added and the samples were placed on the ThermoMixer for overnight digestion (15h) at 37°C and 1000 RPM.

2.8. Denaturation

Previously, 0.5% sodium dodecyl sulfate (SDS) was used to denature the IgG fraction to optimize reduction and alkylation [39]. In this study, 6M guanidine, a potent chaotropic agent, was compared to 0.5% SDS to determine whether an improved signal intensity could be obtained. A mixed standard was used containing 100 mg/L of each therapeutic mAb. Samples were prepared following the procedure described above, with the exception of the denaturation and reduction step which was performed with 50 µL TRIS (100 mM, pH 8.5, 6M guanidine, 20 mM DTT) in triplicate or with 50 µL TRIS (100 mM, pH 8.5, 0.5% SDS, 20 mM DTT) in triplicate, respectively. Relative recovery was calculated as previously mentioned in the AS precipitation procedure.

2.9. Trypsin digestion

Overnight (15h) digestion efficiency of the 7 therapeutic mAbs was examined in relation to the trypsin concentration that was used. QC high was diluted 10 times in human plasma, thereafter 10 µL was pipetted 15 times in a 1 mL LoBind® deep well plate. The samples were prepared as described in the sample preparation section above, with the exception of the trypsin digestion step. Here, 10 µL of varying trypsin concentration (0.25 – 0.5 – 1.0 – 2.0 – 4.0 µg/µL) was used in triplicate. This equals a protein to enzyme (P:E) ratio of 128 – 8, considering plasma can contain as much as 80 µg/µL protein of which 40% are globulins [40].

2.10. Sampling condition

Five different blood collection vacuum tubes were tested to determine whether anticoagulation additives and/or gels had a negative effect on trypsin digestion and/or MS measurements. Blood from a healthy volunteer, collected in various tubes, namely sodium citrate, EDTA, heparin, plain tube, and plain tube with gel, were spiked at a QC high level. After 1 hour, the blood samples were centrifuged at 4000 G for 5 minutes and plasma or serum was transferred to a polypropylene tube. The spiked samples were analyzed according to the sample preparation procedure described above, and the results were assessed with an univariate ANOVA test using Excel 2016 data analysis.

2.11. Method validation

The analytical method was validated in accordance with the EMA guideline for bioanalytical method validation [41]. Method performance was evaluated for linearity, LLOQ, selectivity, carry-over, matrix effect, within-run and between-run accuracy and precision. Stability testing was performed with QC low and high (n=5). Freeze and thaw stability was evaluated for 3 freeze (-80°C) and thaw cycles of 24 hours each. Stability at room temperature (20°C) and 5°C was tested during 3 days. Autosampler stability was tested by reinjecting the QC low and high after 4 days in the autosampler at 10°C. Within-run and between-run accuracy and precision were tested for QC LLOQ, low, med, and high, during 3 days in fivefold. Selectivity was evaluated by analyzing 10 blank human plasma samples. Peaks eluting at the retention time of the signature peptides were integrated and the signal intensity was divided by the LLOQ signal of the respective peaks. Matrix effect was tested by spiking 10 blank human plasma samples at QC low and high level.

2.12. Cross-validation

Vedolizumab EDTA plasma samples, that were collected for therapeutic drug monitoring from our patients as part of their treatment plan, were partitioned in two. One portion was send to an external laboratory where the concentration vedolizumab was quantified using enzyme linked immunosorbent assay (ELISA). The other portion was quantified with the validated LC-MS/MS method. The results from the 22 samples tested with both methods, ranging from 9 to 63 mg/L, were compared using simple linear regression and Bland-Altman with software GraphPad.

3. Results and discussion

3.1. Ammonium sulfate precipitation

The effect of different AS concentrations was examined in relation to the relative recovery of the mAbs. This was done to determine the AS amount that was needed for optimum precipitation. DNX requiring between 39.4 and 44.4% saturated AS was in line with our previous finding where 41.2% saturated AS sufficed [39]. For the majority of the therapeutic mAbs, at least 45% saturated AS was required to reach a maximum recovery. EMZ and DPL were the only exceptions, requiring 50% saturated AS (Fig. 1). Since general properties such as molecular weight are comparable, differences in the ease of precipitation may be explained by differences in comprising amino acids which ultimately determine the isoelectric point (pI) of the protein. AS precipitation was performed at pH 8.5. The mAbs with theoretical pI values close to this pH value have lower solubility and precipitate easier, as their net charge is close to 0. mAbs with lower pI values, such as EMZ with pI 6.44 and DPL with pI 6.74, have higher solubility and precipitate only at higher ammonium sulfate concentration due to the higher number of surface charges. Infliximab with pI 7.5, RTX pI 8.19, DNX pI 8.05, CTX pI 7.68, and VDZ pI 7.55 have, in contrast to EMZ and DPL, a pI closer to the pH of the buffer solution and, therefore, precipitate at a lower AS concentration. At 50% saturated ammonium sulfate, the albumin fraction remained soluble conform our previous finding.

3.2. Sample preparation

A comparison in sample preparation was made between the proposed method described in section 2.5 and the widely used MeOH pellet digestion (section 2.7). Here, similar samples, internal standard, and trypsin volumes were used for both methods to allow for a fair comparison. The AS derived pellet was much smaller compared to the MeOH pellet providing a favorable protein to enzyme (P:E) ratio. Furthermore, the MeOH prepared samples were still opaque in color which is an indication of incomplete digestion. Finally, in contrast to MeOH pellet digestion, the AS method incorporates a washing step prior to digestion which eliminates salts that can reduce trypsin activity. These factors lead to suboptimal digestion in the MeOH prepared samples as can be seen in Fig. 2. The VDZ signature peptide LEWIGEIDPSESNTNYNQK, containing four acidic groups is most affected. This could be due to a combination of factors. First, in contrast to guanidine-ions,

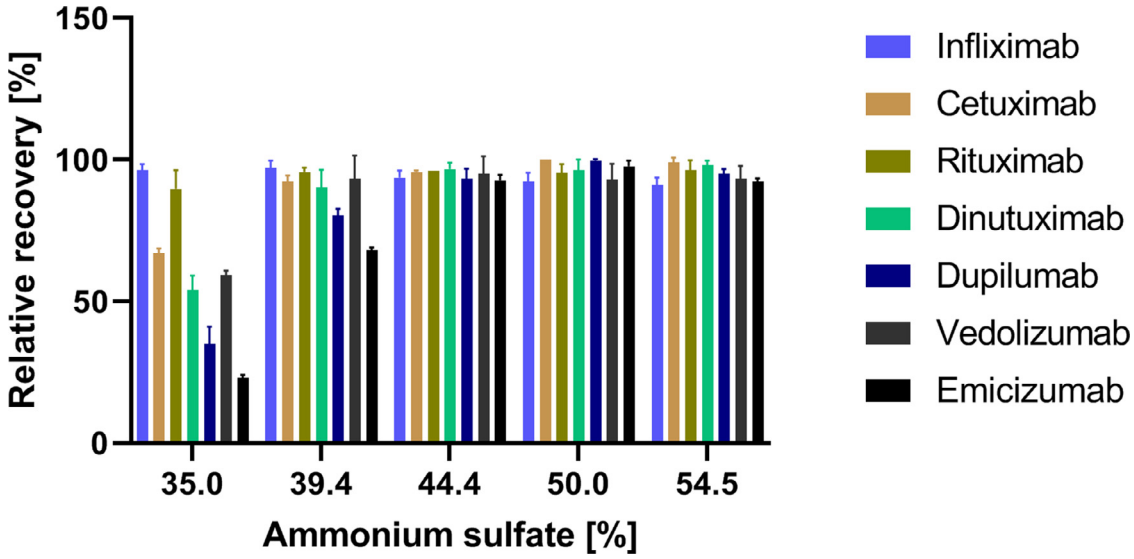

Fig. 1. The effect of ammonium sulfate (AS) concentration on the relative recovery of 7 therapeutic monoclonal antibodies (mAbs). Each data point on the graph consists of the mean and standard deviation (SD) error bar (n=3 for each mAb).

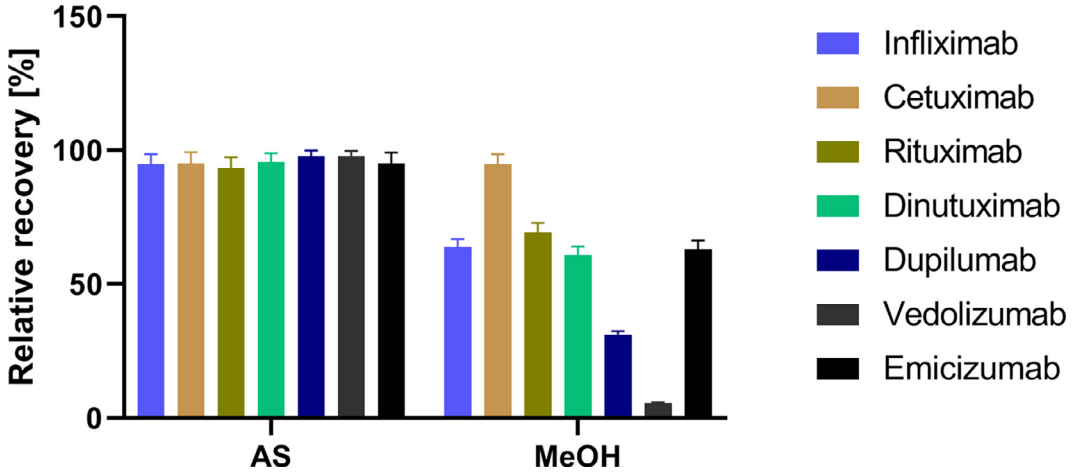

Fig. 2. Relative recovery of 7 therapeutic monoclonal antibodies (mAbs) obtained by using AS guanidine sample preparation and MeOH pellet digestion. Each data point on the graph consists of the mean and standard deviation (SD) error bar (n=5 for each mAb).

methanol is unable to efficiently disturb internal ionic interactions, which could lead to shielding from trypsin digestion. Furthermore, the overall negatively charge peptide, attract trypsin to a lesser extent resulting in a lower digestion efficiency. This is especially important since the MeOH pellet was much larger in size. CTX signature peptide YASESISGIPSR is highly hydrophilic and shows similar recovery between the two sample preparation methods. These hydrophilic groups form weak hydrogen bonds which can easily be broken. Overall, AS in combination with guanidine 6M, and a washing step prior to digestion, showed superior digestion recoveries. The relative signal intensity was on average twice as high with the AS method compared to the widely used MeOH pellet digestion method.

3.3. Denaturation

In contrast to urea, guanidine does not dissociate upon heating. This spares the protein from unwanted side reactions, such as carbamylation adduct reactions that are common with urea reagents. The utilization of guanidine as a substitute for SDS was examined and results showed that for most therapeutic mAbs significantly higher signals were obtained when using guanidine (Fig. 3). IFX and CTX quantifier peptides had similar recoveries in both guanidine and SDS which shows that these peptides are presumably easily accessible by trypsin. Since a stable isotopically labeled IS was used to correct for ionization suppression, the signal gain was due to efficient digestion and not ionization differences between 0.5% SDS or 6M guanidine. Possible explanations are that 0.5% SDS is not as efficient in unfolding and solubilizing the therapeutic mAbs. SDS forms negatively charged SDS-protein complexes, and it reduces the interactions between hydrophobic regions of proteins. Guanidine ions cover hydrophobic surfaces with higher efficiency due to their small size and as chaotropic agent disrupts hydrogen bonds between water molecules around the hydrophobic regions of protein, hereby decreasing the internal hydrophobic interaction. SDS in contrast, is less effective in disturbing the hydrogen bond network between water molecules around protein. Furthermore, SDS molecules with their long hydrophobic tails are less effective in reaching all hydrophobic regions. This was especially evident in hydrophobic signature peptides such as DNX SSSTAYMHLK and RTX ASGYTFTSYNMHWVK where the internal hydrophobic bonds were not efficiently disrupted by SDS leading to inefficient digestion. Furthermore, remnant SDS in the sample solution influences chromatographic retention causing unpredictable

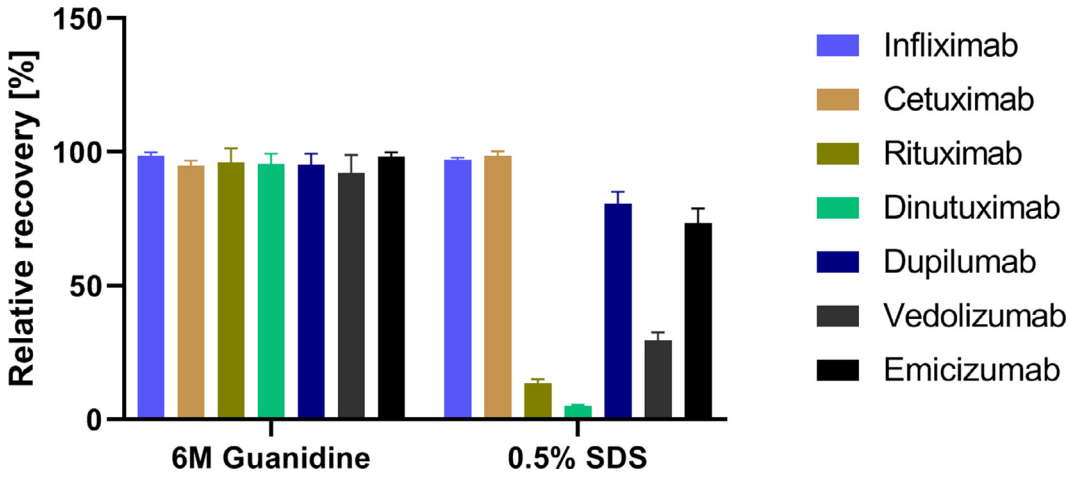

Fig. 3. Relative recovery of 7 therapeutic monoclonal antibodies (mAbs) obtained by using 6M guanidine and 0.5% SDS as a solvent and denaturant. Each data point on the graph consists of the mean and standard deviation (SD) error bar (n=3 for each mAb).

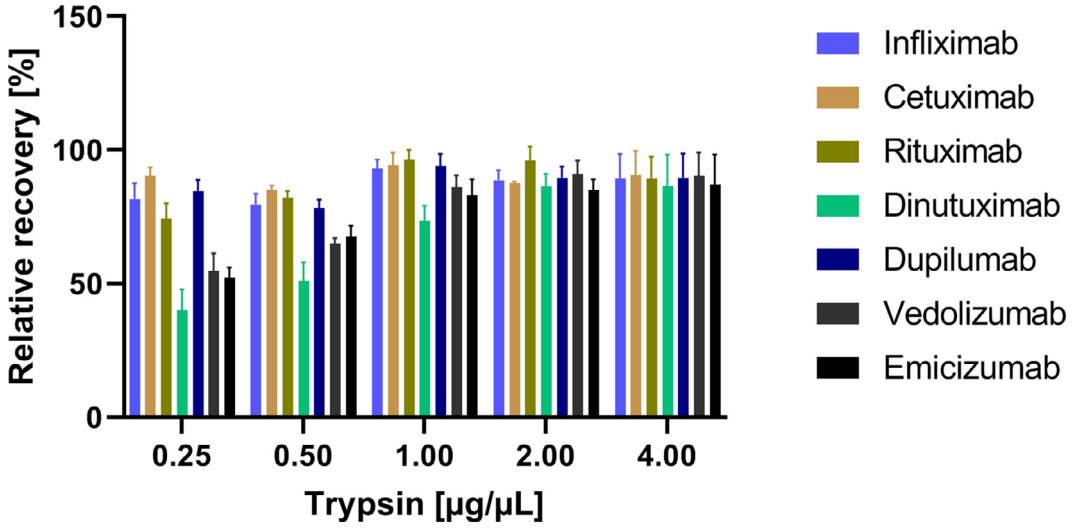

Fig. 4. Effect of the trypsin concentration on the relative recovery of 7 therapeutic monoclonal antibodies (mAbs). Each data point on the graph consists of the mean and standard deviation (SD) error bar (n=3 for each mAb).

retention times, which is highly undesirable especially when targeting multiple components.

3.4. Trypsin digestion

The AS sample preparation method precipitates both endogenous and therapeutic mAbs. The obtained protein pellet needs to be efficiently and reliably digested in order to achieve a high accuracy and precision. The sample preparation method produces a large pellet of approximately 320 µg IgG which was found to require overnight digestion. A varying trypsin concentration was tested to determine the optimum concentration for maximum recovery of the mAbs. At the addition of 10 µL (1 µg/µL) trypsin, all mAbs except DNX (SSSTAYMHLK peptide) were completely digested (Fig. 4). The differences in ease of digestion is partly due to the accessibility of the signature peptide to trypsin, which can be limited due to protein folding and possibly partially due to neighboring amino acids. One of the flanking amino acids for the DNX signature peptide (**D**KSSSTAYMHLKS) was aspartic acid, which is negatively charged at pH 8.5. This negative charge could partially neutralize the positive charge of lysine, which would cause trypsin to be less attracted to the peptide. Nonetheless, at 2 µg/µL trypsin, representing a P:E ratio of 16, a plateau was reached for all therapeutic mAbs.

3.5. Sampling conditions

In this experiment, the effect of 5 different blood collection vacuum tubes on the relative recovery of the mAbs was examined. All collection tubes showed a similar relative recovery with an average CV of only 5.8% between different analytes. (Fig. 5). Univariate ANOVA test provided a P value of 0.17 which indicates that there was no significant difference in relative recovery of the mAbs in plasma and/or serum obtained from different sampling tubes. EDTA or sodium citrate, which are present in plasma collection tubes, binds to calcium ions thus inhibiting coagulation and could interfere with digestion, since calcium protects trypsin from autolysis. However, these small molecules are eliminated in the AS precipitation step, therefore plain tubes and tubes with additives show similar relative recoveries indicating that these additives do not interfere with digestion. Heparin, a known serine protease inhibitor might also inhibit trypsin activity, however due to its high solubility, heparin is presumably eliminated during the AS precipitation step and showed no influence on digestion. Furthermore, the use of gel tubes which could bind or limit the passage of IgG was

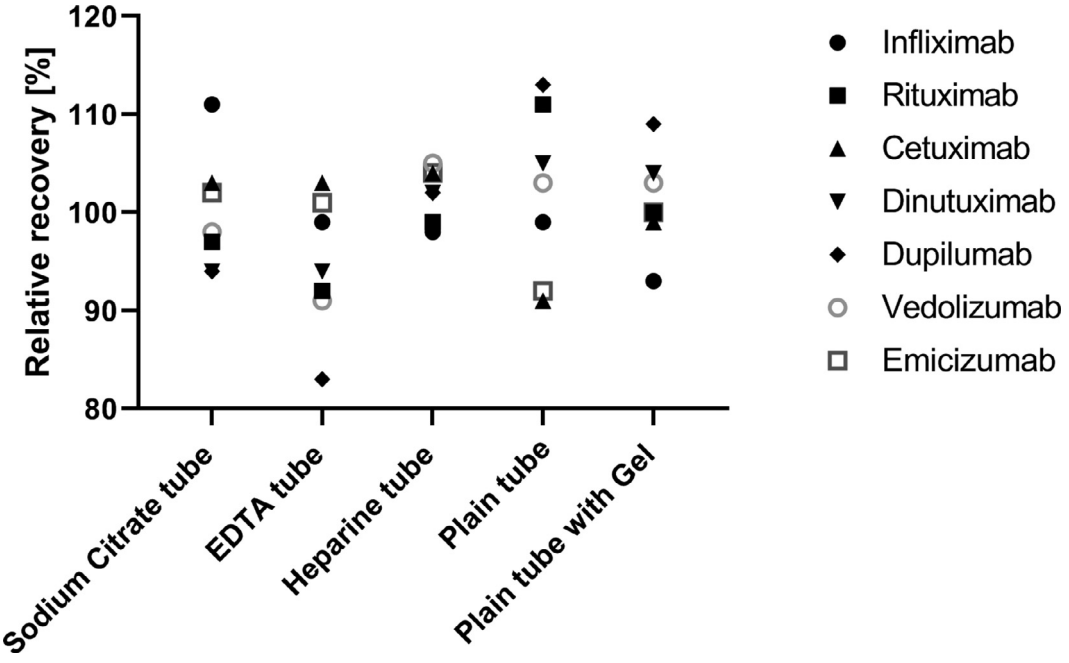

Fig. 5. Five blood collection vacuum tubes spiked with QC high showing the relative recovery results for 7 therapeutic monoclonal antibodies.

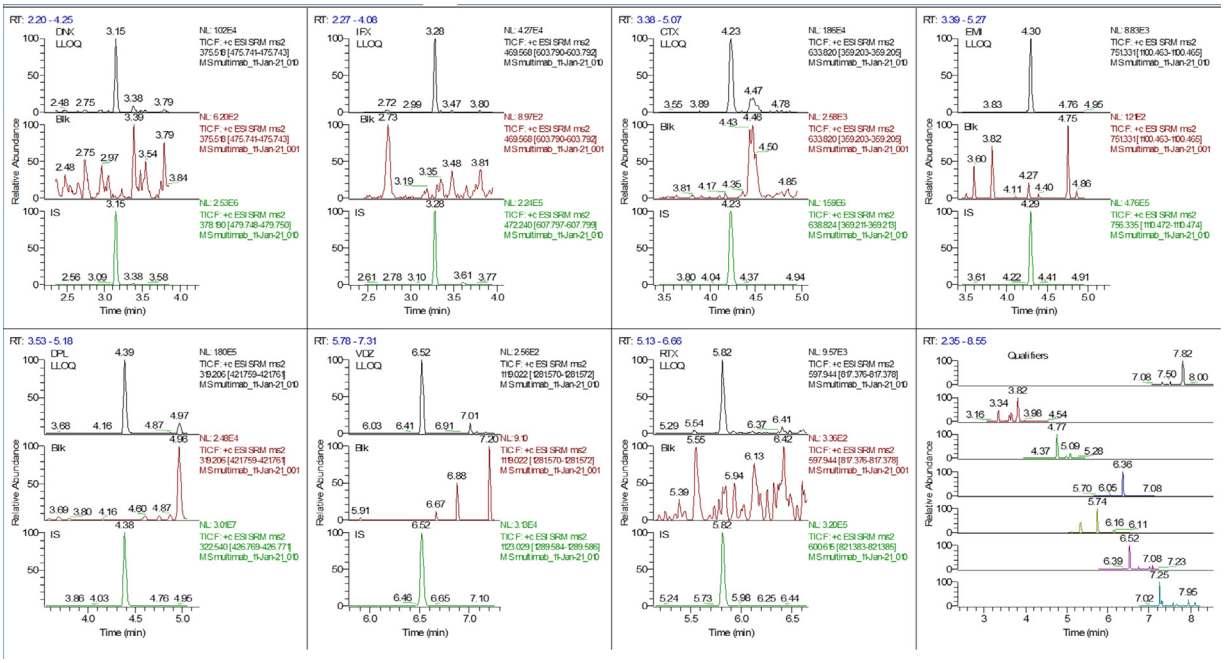

Fig. 6. SRM transitions of quantifier peptides for LLOQ, Blk and internal standard (IS) for 7 therapeutic monoclonal antibodies. Bottom right graph represents SRM transitions of the qualifier peptides, from top to bottom DNX, IFX, CTX, EMZ, DPL, VDZ and RTX.

also investigated and no significant influence on the recovery was observed, which shows that the tested therapeutic mAbs did not bind to this medium.

3.6. Method validation

The analytical method was validated in accordance with the EMA guideline for bioanalytical method validation. The within-run and between-run precision, expressed as relative standard deviation (RSD) for QC Low, Med and High, was for all components <10%, which was in agreement with the requirements of the EMA guideline of 15% (Table 2). Accuracy, expressed in percent bias was also in line with the guideline of <15%. The acceptance criteria for selectivity were met, none of the blanks had a signal greater than >20% of the LLOQ. Linearity for all components was greater than R^2 >0.995 (Table 2). The LLOQ was for all components, within acceptance criteria S/N >5 (Fig. 6).

QC Low for DPL had an RSD of 18.2%, which was greater than the EMA recommendation of RSD <15%. The use of alternative signature peptides was examined to further minimize matrix sensitivity, but unfortunately all candidate peptides did not pass the selectivity criteria due to interfering peaks. DPL could benefit from the use of SIL DPL as an IS to correct for digestion differences, as this was probably the cause of the wider RSD in spike recovery for

Table 2
Validation summary report preformed following EMA guideline for IFX, CTX, RTX, DNX, DPL, VDZ and EMZ.

Validation parameter	QC	Expressed as	IFX	CTX	RTX	DNX	DPL	VDZ	EMZ	Acceptance
Within-run precision	QC LLOQ	RSD [%]	2.3	3.1	6.3	5.8	9.9	12.1	3.7	<20
	QC Low		1.9	1.6	6.8	3.4	7.8	6.8	2.6	<15
	QC Med		1.5	1.0	4.8	3.8	8.2	2.6	2.0	<15
	QC High		1.7	1.1	1.8	2.0	4.1	3.7	1.3	<15
Between-run precision	QC LLOQ	RSD [%]	2.9	2.0	0.8	4.1	5.5	0.0	7.8	<20
	QC Low		2.0	0.0	0.0	2.4	1.1	0.0	2.5	<15
	QC Med		6.0	3.8	3.1	3.0	1.7	3.8	3.4	<15
	QC High		3.4	1.9	6.6	4.9	6.5	5.7	2.5	<15
Accuracy	QC LLOQ	Bias [%]	0	-9.4	-2.8	-4.8	-6.1	-4.5	-6.7	<20
	QC Low		-5.5	-11.3	-1.3	-7.1	-4.3	-6.7	-8.2	<15
	QC Med		-2.0	-4.1	3.0	-0.2	3.8	0.2	-2.3	<15
	QC High		-1.5	-4.2	0.7	-0.7	-1.6	-1.3	-1.4	<15
Selectivity	Blk (HP) n=10	Mean relative to LLOQ [%]	2	1	2	1	0	2	2	<20
LLOQ	QC LLOQ	S/N	53.1	211.3	79.6	119	146	10.8	54	>5 ×
Linearity	Standard 1 - 8	R^2	0.9996	0.9998	0.9997	0.9984	0.9977	0.9989	0.9998	>0.99
Freeze and Thaw	QC Low	Day 3 Bias [%]	-3.8	-11.5	-7.0	-12.1	-10.4	-12.5	-7.1	<15
	QC High		-3.2	-6.5	-2.4	-3.5	-3.1	-2.6	-3.3	<15
Stability 5°C	QC Low	Day 3 Bias [%]	-4.8	-13.6	-4.2	-6.0	-8.5	-8.1	-6.0	<15
	QC High		-4.6	-9.2	-2.7	-5.0	-5.3	-5.2	-5.6	<15
Stability 20°C	QC Low	Day 3 Bias [%]	-5.6	-11.4	-6.5	-7.7	-11.2	-4.9	-3.7	<15
	QC High		-4.0	-6.3	3.7	1.0	6.0	-0.1	-3.6	<15
Matrix effect spike recovery	Spike low n=10	RSD [%]	4.1	3.0	9.9	14.3	18.2	8.0	3.6	<15
	Spike high n=10	RSD [%]	1.5	1.5	8.0	9.9	11.1	7.7	2.3	<15
Carry-over	Blk after Std 8	Relative to LLOQ [%]	1	1	2	0	1	0	0	<20

HP = Human plasma
Blk = Blank
QC = Quality control
RSD = Relative standard deviation
S/N = Signal to Noise ratio
IFX= Infliximab, CTX = Cetuximab, RTX = Rituximab, DNX = Dinutuximab, DPL = Dupilumab, VDZ = Vedolizumab and EMZ = Emicizumab

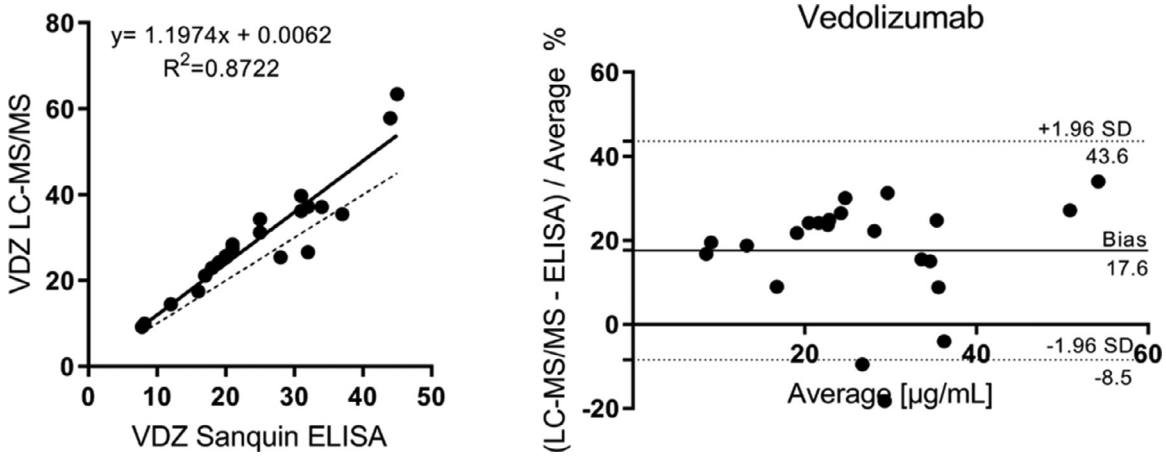

Fig. 7. Vedolizumab cross-validation showing simple linear regression (A), and Bland-Altman plot (B) with n=22.

QC Low. For this method, a higher error than desired was accepted for DPL since no alternative methods are available. Matrix effect for the remaining therapeutic mAbs was in concordance with the guideline (Table 2). Freeze and thaw during 3 cycles, showed no decrease in concentration. Furthermore, QC Low and High were stable during 3 days at 5°C and 20°C.

3.7. Cross-validation

22 human vedolizumab EDTA plasma samples routinely obtained from our patients as part of their TDM treatment plan were analyzed on both ELISA and LC-MS/MS. Simple linear regression showed a good agreement between methods with an R^2=0.87 (Fig. 7A). Bland-Altman shows a bias of 17.6% (Fig. 7B) which is in line with previous ELISA, LC-MS/MS comparisons [21]. LC-MS/MS results are higher compared to ELISA. This could be due to the ability of the LC-MS/MS to measure all fractions free and total, whereas the sandwich type ELISA assay relies on immunoaffinity interaction between the two free paratopes on vedolizumab. Both paratopes are required for measurement, one paratope is used for binding vedolizumab to the 96 well plate and the other to the detecting antibody. Since vedolizumab with only one free paratope is not measured with ELISA, the assay potentially underestimates the concentration of active vedolizumab.

4. Conclusion

A generic sample preparation method was developed to simultaneously quantitate 7 therapeutic mAbs (INF, RTX, CTX, DPL, DNX, VDZ, EMZ) in human plasma. AS precipitation, denaturation and trypsin digestion steps were optimized before analytical method validation. The use of 50% saturated AS provided maximum recovery for all examined mAbs, and digestion efficiency was greatly improved by using 6M guanidine as a protein solvent and denaturant.

The method uses readily available reagents and consumables and has a fast and easy sample preparation. Furthermore, SIL peptides were used as IS, instead of SIL mAbs which are often commercially unavailable for most therapeutic mAbs.

The AS derived pellet was much smaller than the methanol pellet and is thus able to removed more plasma proteins that otherwise could interfere with digestion, chromatographic separation or ionization. Furthermore, in contrast to methanol precipitation, the AS pellet is easily dissolved in buffer, which allows for faster centrifugation speeds to be used and making decanting of the supernatant possible. Due to the complete protein unfolding and solubilization with guanidine, reduction and alkylation steps are efficiently performed allowing improved trypsin digestion and a higher recovery.

The method was validated in accordance with the EMA guidelines and all acceptance criteria were met. Only DPL showed to have a higher (18.2%) than acceptable (<15%) RSD for the matrix effect for the low concentration. However, overall the method performed extremely well. The mAbs were stable at both 5°C and 20°C for 3 days. Five different blood sampling vacuum tubes were examined and all showed to provide the same result. The method is suitable for use in clinical studies and for TDM and can in time be expanded with other therapeutic mAbs.

Informed consent

Samples were drawn after obtaining informed consent

Ethics committee

The use of anonymized remnant material drawn as part of the treatment protocol and with patient's informed consent was in accordance with University Medical Center Utrecht policy and ethical standards.

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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2021.462489.

CRediT authorship contribution statement

Mohsin El Amrani: Writing – review & editing. **Laszlo Gerencser:** Data curation. **Alwin D.R. Huitema:** Writing – review & editing. **C. Erik Hack:** Writing – review & editing. **Matthijs van Luin:** Writing – review & editing. **Kim C.M. van der Elst:** Writing – review & editing.

References

[1] A. Mullard, FDA drug approvals, Nat. Rev. Drug Discov. 16 (2) (2016) 73–76 2017https://doi.org/10.1038/nrd.2017.14.

[2] H.A. Lagasse, A. Alexaki, V.L. Simhadri, N.H. Katagiri, W. Jankowski, Z.E. Sauna, C. Kimchi-Sarfaty, Recent Advances in (therapeutic protein) Drug Development, 113, 2017 F1000Res 6https://doi.org/10.12688/f1000research.9970.1.

[3] K.J. Bronsema, R. Bischoff, N.C. van de Merbel, Internal standards in the quantitative determination of protein biopharmaceuticals using liquid chromatography coupled to mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 893 (894) (2012) 1–14 https://doi.org/10.1016/j.jchromb.2012.02.021.

[4] S. Marchetti, J.H. Schellens, The impact of FDA and EMEA guidelines on drug development in relation to Phase 0 trials, Br. J. Cancer 97 (5) (2007) 577–581 https://doi.org/10.1038/sj.bjc.6603925.

[5] B. An, M. Zhang, J. Qu, Toward sensitive and accurate analysis of antibody biotherapeutics by liquid chromatography coupled with mass spectrometry, Drug Metab. Dispos. 42 (11) (2014) 1858–1866 https://doi.org/10.1124/dmd.114.058917.

[6] J.W. Lee, M. Kelley, L.E. King, J. Yang, H. Salimi-Moosavi, M.T. Tang, J.F. Lu, J. Kamerud, A. Ahene, H. Myler, C. Rogers, Bioanalytical approaches to quantify "total" and "free" therapeutic antibodies and their targets: technical challenges and PK/PD applications over the course of drug development, AAPS J. 13 (1) (2011) 99–110 https://doi.org/10.1208/s12248-011-9251-3.

[7] I. Onami, M. Ayabe, N. Murao, M. Ishigai, A versatile method for protein-based antigen bioanalysis in non-clinical pharmacokinetics studies of a human monoclonal antibody drug by an immunoaffinity liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1334 (2014) 64–71 https://doi.org/10.1016/j.chroma.2014.01.082.

[8] O. Heudi, S. Barteau, D. Zimmer, J. Schmidt, K. Bill, N. Lehmann, C. Bauer, O. Kretz, Towards absolute quantification of therapeutic monoclonal antibody in serum by LC-MS/MS using isotope-labeled antibody standard and protein cleavage isotope dilution mass spectrometry, Anal. Chem. 80 (11) (2008) 4200–4207 https://doi.org/10.1021/ac800205s.

[9] F. Osaki, K. Tabata, T. Oe, Quantitative LC/ESI-SRM/MS of antibody biopharmaceuticals: use of a homologous antibody as an internal standard and three--step method development, Anal. Bioanal. Chem. 409 (23) (2017) 5523–5532 https://doi.org/10.1007/s00216-017-0488-2.

[10] J.W. Lee, Generic method approaches for monoclonal antibody therapeutics analysis using both ligand binding and LC-MS/MS techniques, Bioanalysis 8 (1) (2016) 19–27 https://doi.org/10.4155/bio.15.231.

[11] R. Bischoff, K.J. Bronsema, N.C. van de Merbel, K.J. Bronsema, N.C. van de Merbel, Analysis of biopharmaceutical proteins in biological matrices by LC-MS/MS I. Sample preparation, TrAC Trends Anal. Chem. 48 (2013) 41–51 Supplement Chttps://doi.org/https://doi.org/10.1016/j.trac.2012.11.015.

[12] P. Bults, N.C. van de Merbel, R. Bischoff, Quantification of biopharmaceuticals and biomarkers in complex biological matrices: a comparison of liquid chromatography coupled to tandem mass spectrometry and ligand binding assays, Expert Rev. Proteomics 12 (4) (2015) 355–374 https://doi.org/10.1586/14789450.2015.1050384.

[13] I. van den Broek, W.D. van Dongen, LC-MS-based quantification of intact proteins: perspective for clinical and bioanalytical applications, Bioanalysis 7 (15) (2015) 1943–1958 https://doi.org/10.4155/bio.15.113.

[14] P.M. Ladwig, D.R. Barnidge, M.A.V. Willrich, Mass spectrometry approaches for identification and quantitation of therapeutic monoclonal antibodies in the clinical laboratory, Clin. Vaccine Immunol. 24 (5) (2017) https://doi.org/10.1128/CVI.00545-16.

[15] M. El Amrani, M.P. van den Broek, C. Gobel, 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.

[16] I. van den Broek, W.M. Niessen, W.D. van Dongen, Bioanalytical LC-MS/MS of protein-based biopharmaceuticals, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 929 (2013) 161–179 https://doi.org/10.1016/j.jchromb.2013.04.030.

[17] F. Li, D. Fast, S. Michael, Absolute quantitation of protein therapeutics in biological matrices by enzymatic digestion and LC-MS, Bioanalysis 3 (21) (2011) 2459–2480 https://doi.org/10.4155/bio.11.237.

[18] 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 (5) (2017) 1195–1205 https://doi.org/10.1007/s00216-016-0045-4.

[19] S. Kaur, L. Liu, D.F. Cortes, J. Shao, R. Jenkins, W.R. Mylott Jr., K. Xu, Validation of a biotherapeutic immunoaffinity-LC-MS/MS assay in monkey serum: 'plug-and-play' across seven molecules, Bioanalysis 8 (15) (2016) 1565–1577 https://doi.org/10.4155/bio-2016-0117.

[20] K. Todoroki, T. Nakano, Y. Eda, K. Ohyama, H. Hayashi, D. Tsuji, J.Z. Min, K. Inoue, N. Iwamoto, A. Kawakami, Y. Ueki, K. Itoh, T. Toyo'oka, Bioanalysis of bevacizumab and infliximab by high-temperature reversed-phase liquid chromatography with fluorescence detection after immunoaffinity magnetic purification, Anal. Chim. Acta 916 (2016) 112–119 https://doi.org/10.1016/j.aca.2016.02.029.

[21] 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 (5) (2019) 640–647 https://doi.org/10.1097/FTD.0000000000000633.

[22] N. Iwamoto, A. Shimomura, K. Tamura, A. Hamada, T. Shimada, LC-MS bioanalysis of Trastuzumab and released emtansine using nano-surface and molecular-orientation limited (nSMOL) proteolysis and liquid-liquid partition in plasma of Trastuzumab emtansine-treated breast cancer patients, J. Pharm. Biomed. Anal. 145 (2017) 33–39 https://doi.org/10.1016/j.jpba.2017.06.032.

[23] H. Liu, A.V. Manuilov, C. Chumsae, M.L. Babineau, E. Tarcsa, Quantitation of a recombinant monoclonal antibody in monkey serum by liquid chromatography-mass spectrometry, Anal. Biochem. 414 (1) (2011) 147–153 https://doi.org/10.1016/j.ab.2011.03.004.

[24] H. Li, R. Ortiz, L. Tran, M. Hall, C. Spahr, K. Walker, J. Laudemann, S. Miller, H. Salimi-Moosavi, J.W. Lee, General LC-MS/MS method approach to quantify therapeutic monoclonal antibodies using a common whole antibody internal

standard with application to preclinical studies, Anal. Chem. 84 (3) (2012) 1267–1273 https://doi.org/10.1021/ac202792n.
[25] H. Li, R. Ortiz, L.T. Tran, H. Salimi-Moosavi, J. Malella, C.A. James, J.W. Lee, Simultaneous analysis of multiple monoclonal antibody biotherapeutics by LC-MS/MS method in rat plasma following cassette-dosing, AAPS J. 15 (2) (2013) 337–346 https://doi.org/10.1208/s12248-012-9435-5.
[26] 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 (28) (2017) 6583–6593 https://doi.org/10.1007/s00216-017-0607-0.
[27] M. Fernandez Ocana, I.T. James, M. Kabir, C. Grace, G. Yuan, S.W. Martin, H. Neubert, Clinical pharmacokinetic assessment of an anti-MAdCAM monoclonal antibody therapeutic by LC-MS/MS, Anal. Chem. 84 (14) (2012) 5959–5967 https://doi.org/10.1021/ac300600f.
[28] A.J. Kleinnijenhuis, M. Ingola, J.H. Toersche, F.L. van Holthoon, W.D. van Dongen, Quantitative bottom up analysis of infliximab in serum using protein A purification and integrated muLC-electrospray chip IonKey MS/MS technology, Bioanalysis 8 (9) (2016) 891–904 https://doi.org/10.4155/bio-2015-0015.
[29] H. Jiang, J. Zeng, C. Titsch, K. Voronin, B. Akinsanya, L. Luo, H. Shen, D.D. Desai, A. Allentoff, A.F. Aubry, B.S. Desilva, M.E. Arnold, Fully validated LC-MS/MS assay for the simultaneous quantitation of coadministered therapeutic antibodies in cynomolgus monkey serum, Anal. Chem. 85 (20) (2013) 9859–9867 https://doi.org/10.1021/ac402420v.
[30] M.T. Furlong, Z. Ouyang, S. Wu, J. Tamura, T. Olah, A. Tymiak, M. Jemal, A universal surrogate peptide to enable LC-MS/MS bioanalysis of a diversity of human monoclonal antibody and human Fc-fusion protein drug candidates in pre-clinical animal studies, Biomed. Chromatogr. 26 (8) (2012) 1024–1032 https://doi.org/10.1002/bmc.2759.
[31] Q. Zhang, D.S. Spellman, Y. Song, B. Choi, N.G. Hatcher, D. Tomazela, M. Beaumont, M. Tabrizifard, D. Prabhavalkar, W. Seghezzi, J. Harrelson, K.P. Bateman, Generic automated method for liquid chromatography-multiple reaction monitoring mass spectrometry based monoclonal antibody quantitation for preclinical pharmacokinetic studies, Anal. Chem. 86 (17) (2014) 8776–8784 https://doi.org/10.1021/ac5019827.
[32] Z. Ouyang, M.T. Furlong, S. Wu, B. Sleczka, J. Tamura, H. Wang, S. Suchard, A. Suri, T. Olah, A. Tymiak, M. Jemal, Pellet digestion: a simple and efficient sample preparation technique for LC-MS/MS quantification of large therapeutic proteins in plasma, Bioanalysis 4 (1) (2012) 17–28 https://doi.org/10.4155/bio.11.286.
[33] G. Liu, Q.C. Ji, R. Dodge, H. Sun, D. Shuster, Q. Zhao, M. Arnold, Liquid chromatography coupled with tandem mass spectrometry for the bioanalysis of proteins in drug development: practical considerations in assay development and validation, J. Chromatogr. A 1284 (2013) 155–162 https://doi.org/10.1016/j.chroma.2013.02.016.
[34] 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 (1) (2017) 2714 https://doi.org/10.1038/s41598-017-02821-x.
[35] C. Gong, N. Zheng, J. Zeng, A.F. Aubry, M.E. Arnold, Post-pellet-digestion precipitation and solid phase extraction: a practical and efficient workflow to extract surrogate peptides for ultra-high performance liquid chromatography–tandem mass spectrometry bioanalysis of a therapeutic antibody in the low ng/mL range, J. Chromatogr. A 1424 (2015) 27–36 https://doi.org/10.1016/j.chroma.2015.10.049.
[36] M.L.P.O.S.N.a.E. ChambersLC-MS Quantification of Trastuzumab Using Pellet Digestion and ProteinWorks eXpress Direct Digest Kits, 2016 http://www.waters.com/webassets/cms/library/docs/720005574en.pdf (Accessed November 2017).
[37] G. Liu, Y. Zhao, A. Angeles, L.L. Hamuro, M.E. Arnold, J.X. Shen, A novel and cost effective method of removing excess albumin from plasma/serum samples and its impacts on LC-MS/MS bioanalysis of therapeutic proteins, Anal. Chem. 86 (16) (2014) 8336–8343 https://doi.org/10.1021/ac501837t.
[38] M.A. 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 (1) (2015) 513–520 https://doi.org/10.1016/j.intimp.2015.07.007.
[39] 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. (2018) https://doi.org/10.1007/s00216-018-1198-0.
[40] M. Leeman, J. Choi, S. Hansson, M.U. Storm, L. Nilsson, Proteins and antibodies in serum, plasma, and whole blood-size characterization using asymmetrical flow field-flow fractionation (AF4), Anal. Bioanal. Chem. 410 (20) (2018) 4867–4873 https://doi.org/10.1007/s00216-018-1127-2.
[41] E.M. AgencyGuideline on Bioanalytical Method Validation, 2011 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf (Accessed 16-October 2017).