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Short communication

Is hydrophobic interaction chromatography the most suitable technique to characterize site-specific antibody-drug conjugates?

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ABSTRACT

Antibody drug conjugates (ADCs) belong to the fastest growing class of therapeutic agents for cancer therapy. In preclinical and clinical studies, there is a significant number of site-specific ADCs (also known as third generation ADCs), which are more homogeneous than their previous generations. These new ADC formats, in which the inter-chain disulphide bridges (hinge cysteines) are not reduced, also need to be deeply characterized. In particular, various quality attributes (QAs) have to be determined, such as free antibody level, average drug to antibody ratio (DAR) and drug distribution. In this contribution, a non-commercial site-specific conjugated ADC has been analyzed by RPLC. Our results demonstrated that RPLC has a huge potential to determine QAs and can replace the historically used HIC methods as RPLC provides better separation quality for such type of ADCs. Site-specific ADCs can be analyzed in RPLC at intact level without the need for sample preparation. A further advantage of RPLC is that it enables the direct coupling to MS and thus allows the fine identification of all eluting species.

1. Introduction

Antibody-drug conjugates represent a promising class of therapeutic agents with a significant potential for cancer treatment. An ADC consists of a recombinant monoclonal antibody (mAb) covalently linked to a cytotoxic molecule (known as payload) using synthetic, labile linkers. ADC products combine the high selectivity and excellent pharmacokinetics of mAbs, together with the anti-tumour potency of the highly cytotoxic payload. As illustrated in Fig. 1, there are mostly three different types of ADC products currently available on the market or applied in clinical trials [1].

In the first ADC type (lysine conjugation), the cytotoxic drugs are randomly attached *via* a cleavable linker to lysine side chains amine residues at the surface of the mAb. It is important to note that mAbs have more than 60 surface exposed lysine residues available for the conjugation [2]. Therefore, although the number of drug molecules incorporated *per* antibody is generally comprised between 0 and 8 (as shown on the drug to antibody ratio (DAR) distribution reported in Fig. 1), lysine conjugation yields a highly heterogeneous population. Due to their complex structure and heterogeneity, the characterization of this ADC type represents a significant challenge and these lysine-conjugated ADC species can only be poorly separated by chromatography due to their high degree of heterogeneity [3].

The second ADC type (cysteine conjugation) uses the cysteines of the reducible inter-chains disulphide bridges located between the two heavy chains or between the heavy and light chains. This conjugation process produces a more homogenous population than the lysine conjugation [4]. Several antibodies having different number of drugs *per* antibody (DAR) are observed (from 0 to 8 drug molecules per antibody, but always with an even number of drugs, as shown in Fig. 1). Despite this, the average DAR can be controlled, and is equal to about 4 in the case of brentuximab vedotin (Adcetris) [5]. The population of drug-to-antibody species can be easily measured in QC labs by hydrophobic interaction chromatography (HIC), which is considered today as the gold standard technique for studying drug distribution, naked antibody content, and average DAR [6,7] of cysteine-conjugated ADC products [8].

Finally, in the latest generation of ADC (site-specific conjugation), the payloads are attached to defined positions that are suitable for drug conjugation. This allows improving the therapeutic index, thanks to higher potency and lower toxicity [9]. Today, more than 40 site-specific drug conjugate technologies have been developed [1]. As example, more homogeneous drug conjugates can be obtained when developing ADCs with additional cysteines engineered into different sites of the mAb, that have different

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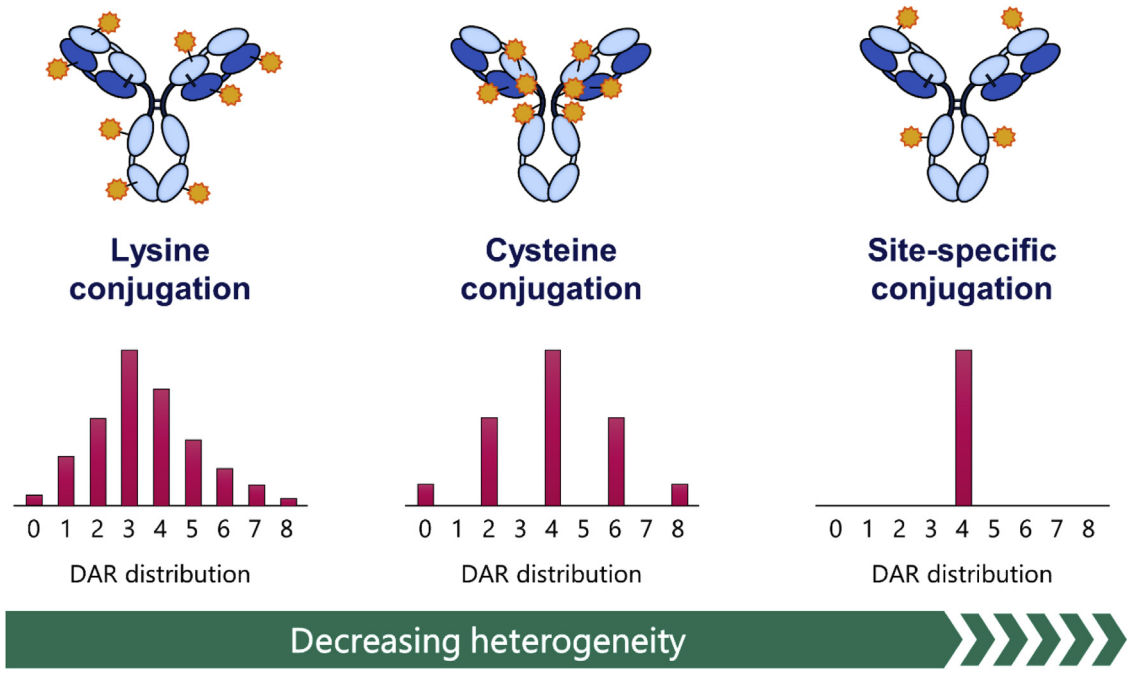

Fig. 1. Schematic description of the first (lysine conjugation), second (hinge cysteine conjugation) and third (site-specific conjugation) types of ADC products. The theoretical DAR distribution of the three ADC types was also highlighted.

solvent accessibility and local charges [10,11]. These products theoretically have a near-uniform stoichiometry of cytotoxic molecules attached per antibody molecule without disruption of inter-chain disulphide bonds (average DAR of near 2 or 4, depending on the number of engineered cysteines *per* antibody molecules, as illustrated in Fig. 1). However, in practice, the DAR distribution of these ADCs can be more heterogeneous, due to differences in payload accessibility to the mAb structure and possible artefacts during ADC synthesis (protecting groups not cleaved) [12]. Even if site-specific ADCs have a simplified composition, they still require strict control during manufacturing to ensure batch-to-batch reproducibility.

To the best of our knowledge, HIC is still considered in the industry as the method of choice for the analysis of intact site-specific ADCs under non-denaturing conditions. In this contribution, we highlight that RPLC can provide a better solution than HIC for the analysis of intact site-specific ADCs, including the determination of average DAR.

2. Experimental

2.1. Reagents and materials

Water and acetonitrile (ACN) were UHPLC-MS grade and purchased from Biosolve (Valkenswaald, Netherlands). Trifluoroacetic acid (TFA, > 99%), formic acid (FA, > 99%), ammonium sulfate, sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Sigma–Aldrich (Buchs, Switzerland). Rapid® PNGase F (non-reducing format) enzyme was purchased from New England Biolabs Inc. (Ipswich, MA, USA). Reaction buffer for Rapid® PNGase F deglycosylation was provided together with the enzyme.

Site-specific cysteine conjugated monoclonal antibody drug conjugate was kindly provided by Novartis Pharma AG (Basel, Switzerland).

2.2. Sample preparation

N-deglycosylation of ADC product was performed by incubating 15.2 μL of site-specific cysteine linked ADC JBH-492 (16.47 μg/μL), 24.8 u L of water and 8 μL of "5X Rapid PNGase F Buffer" at 75 °C for 5 min. Then, 2 μL Rapid PNGase F enzyme was added and the mixture was incubated at 50 °C for 10 min. Final sample concentration was 5 μg/μL.

2.3. Instrumentation and columns

LC measurements were performed using a Waters Acquity UPLC™ system equipped with a binary solvent delivery pump, an auto-sampler and fluorescence (FL) detector. Data acquisition and instrument control were performed by Empower Pro 2 software (Waters). Retention and resolution modeling was performed with DryLab® 4 software (Molnár-Institute, Berlin, Germany).

RPLC-MS analyses were acquired with an ACQUITY UPLC system (Waters, Milford, MA, USA) equipped with a binary pumping system, a fixed loop injector, and coupled to a fluorescence detector (FD) and an electrospray time-of-flight mass spectrometer (Waters Xevo™ Q-ToF) operating in the positive ion mode. Capillary voltage was set to 3.0 kV, sample cone voltage to 30 V, source temperature to 150 °C, desolvation gas temperature to 500 °C and gas flow to 1000 L/h. Acquisitions were performed in the *m/z* range of 500–5500 with a 1 s scan time. The instrument was calibrated using the singly charged ions produced by a 2 g/L sodium iodide solution in 2-propanol/water (50/50 v/v). Data acquisition and analysis were performed with MassLynx 4.1 (Waters). Deconvolution was performed by manual identification of multiply charged series components, by setting 0.1 Da as the resolution between data points when the electrospray spectra were transformed onto a molecular mass axis. Lowest/highest molecular mass, which the algorithm considered for the peak series were set to 145–155 kDa.

MAbPac RP 2.1 mm x 100 mm (4 μm) and MAbPac HIC-10 4.6 mm x 100 mm (5 μm) columns were purchased from Thermo Fisher Scientific AG (Reinach, Switzerland). BioResolve RP mAb Polyphenyl 2.1 × 100 mm (2.7 μm) column was kindly provided by Waters (Milford, MA, USA).

2.4. Chromatographic conditions

For the RPLC measurements, the mobile phase "A" consisted of 0.05% TFA + 0.05% FA in water, whereas the mobile phase "B" was 0.05% TFA + 0.05% FA in ACN. Preliminary studies were performed to evaluate the impact of the TFA/FA ratio on chromatographic performance with the purpose to decrease the TFA concentration as much as possible to improve MS sensitivity. Linear gradients were run from 25 to 50% B with different gradient times (steepness) at a flow rate of 0.3 mL/min. Based on the initial runs, resolution maps were generated by retention modeling software and the gradient program was optimized. A gradient from 30 to 46% B within 10 min provided the highest resolution and was thus applied for our experiments. Recovery and possible on-column degradation of mAb and ADC samples may depend on mobile phase temperature [4,13], and therefore its impact was also studied. It was found that working at T = 60 °C gives appropriate recovery and peak shape, moreover no sample degradation was observed within the analysis time at such temperature. Fluorescence detection was carried out at excitation of 280 nm and emission at 350 nm. Sample injection volume was 1 μL. The RPLC conditions were developed on the MAbPac RP column, but the BioResolve RP mAb Polyphenyl offered the same quality of separation and could thus be considered as an alternative column.

For the HIC measurements, the mobile phase "A" consisted of 1.5 M ammonium sulfate containing 0.1 M phosphate buffer, while mobile phase "B" was 0.1 M phosphate buffer. The mobile phase pH was set to pH = 7, by adjusting the ratio of sodium dihydrogen phosphate and disodium hydrogen phosphate. The separations were performed on the MAbPac HIC-10 column operating at 1 mL/min flow rate. First, linear gradients were run from 0 to 100% B at different gradient steepness and then, the gradient program was optimized on the basis of retention modeling. It was found that a gradient from 25 to 75% B provides the best separation. To be comparable with the RPLC separation, a gradient time of $tG = 10$ min was finally set. Ambient mobile phase temperature was applied and 2 μL sample was injected.

3. Results and discussion

3.1. RPLC vs. HIC

Among the various liquid chromatographic modes, HIC is considered as the reference technique to separate the different populations of ADC molecules that differ in the number of drugs per antibody (DAR) species [14,15]. By using HIC, both the average DAR and the DAR distribution can be determined for conventional cysteine linked ADCs [4,7,8,16–18]. Alongside HIC, RPLC can also be a powerful tool for the separation of DAR species of reduced ADC samples (light chain and heavy chain related species) [4,19]. The main difference between HIC and RP is that proteins maintain their native structure via intra-molecular forces in HIC conditions, while they are denaturated under RP conditions. Since RPLC employs harsh conditions (i.e. mobile phase with acidic additive, organic modifier, and elevated temperature) there is also a risk of on-column degradation. In this context, HIC is routinely used [20], and its industrial significance has been growing. Indeed, HIC is considered the best option for the analysis of conventional cysteine linked ADCs, as the non-covalently connected mAbs (and ADCs) preserve their conformation and do not dissociate into their subunits (heavy and light chains). On the other hand, in many cases HIC does not have sufficient resolution to separate positional isomers at the protein level, and due to the high mobile phase salt concentration, MS hyphenation is only feasible with 2D-LC by using a desalting step for analysis in the second dimension [21]. Because of the limited kinetic efficiency of HIC, the separation of other than native cysteine linked ADC species is challenging. In this study, our purpose was to illustrate the benefits of RPLC compared to HIC when analysing heterogeneous population of a site-specific cysteine conjugated ADC. The aim of the developed method was: (1) separation of the unconjugated mAb from the ADC species, (2) separation of the DAR species, to determine average DAR and DAR distribution, and (3) separation of positional isomers or DAR variants – if possible.

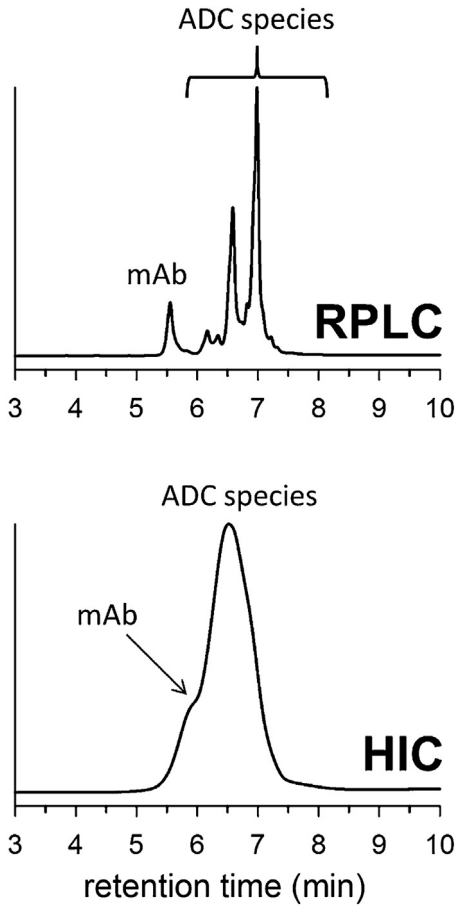

Fig. 2. RPLC and HIC separation of site-specific cysteine linked ADC sample spiked with 10% unconjugated mAb. Chromatographic conditions are described in Section 2.4.

The same method development approach was used for both RPLC and HIC. After a few preliminary (screening) measurements, the gradient programs were systematically optimized using retention modelling software. Same column length (10 cm), gradient time (10 min) and retention window were considered. Fig. 2 shows the chromatograms obtained by injecting a spiked ADC sample (containing ~ 10% unconjugated mAb). In HIC conditions, all the ADC species co-elute and the unconjugated mAb can only be partially resolved (eluting in the front shoulder of the main peak). Conversely, in RP conditions the unconjugated mAb can be baseline separated from the early eluting DAR species. Moreover, the DAR2, DAR3 and DAR4 species can also be discriminated (see the peaks identification in Section 3.2) and their variants were partly resolved too. The average DAR (DAR_{av}) can be determined only on the basis of the RPLC measurement (impossible in HIC), and was equal to 3.5. DAR distribution can also be measured, which is generally not the case for conventional cysteine linked ADCs that are analysed mostly at sub-unit level (reduced ADC) in RPLC. Here - thanks to the intact level analysis – 7.4% DAR2, 37% DAR3 and 55.6% DAR 4 were found. It has to be mentioned that in our ADC sample, the linker and payload were connected *via* a disulphide bridge, therefore a conventional

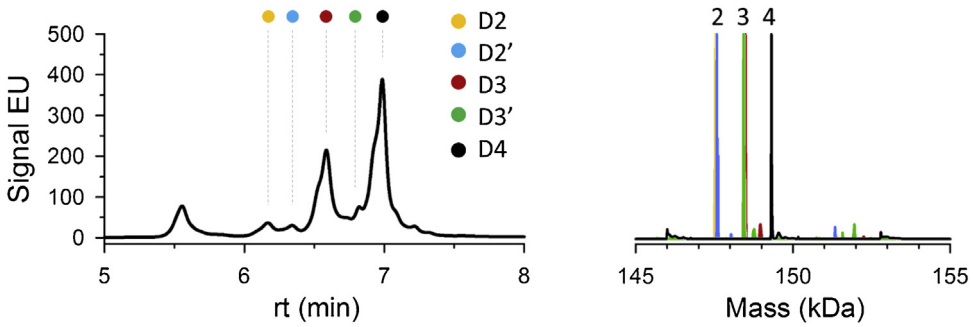

Fig. 3. RPLC-MS analysis of site-specific cysteine linked ADC sample spiked with 10% unconjugated mAb, with associated peak assignment (left panel) and overlay of the deconvoluted mass spectra (right panel) of each variant loaded with up to 4 drug payloads.

RPLC analysis performed on reduced sample would not make sense, since it would also induce the detachment of the drug payload and thus a biased evaluation of the DAR distribution. In addition, RPLC conditions should be *a priori* as mild as possible (low temperature) and within the appropriate limit to avoid protein adsorption on the stationary phase. Indeed, in many cases the recovery of intact mAb and ADC species was not appropriate at temperature lower than 60 °C [22]. Therefore, the effect of mobile phase temperature on recovery and degradation was studied between 50 and 80 °C in 5 °C steps. No degradation was observed in the entire temperature range (please note that gradient time was set to 10 min, therefore the exposure time was limited). However, at temperatures lower than 60 °C, the recovery of both mAb and ADC dropped significantly, as compared to the peak area obtained at 80 °C. At 60 °C, more than 98% recovery was observed for all species, and therefore 60 °C was selected as mobile phase temperature (probably 65 °C would provide very similar results and therefore would also be a good choice).

Various proportions of TFA and FA ranging from 0.1%TFA to 0.01%TFA/0.09%FA were studied to improve MS sensitivity. 0.1% TFA is a commonly used additive for the analytical characterization of proteins in RPLC, since protein-TFA ion-pair behaviour results in desirable chromatographic properties, even though MS ionization could be drastically supressed by TFA [23]. In our study, we found that the combination of 0.05% TFA + 0.05% FA gave very similar elution profiles and comparable resolution as what can be obtained with 0.1% TFA. Below 0.05% TFA, the resolution between DAR species decreased significantly. Finally, the RP stationary phases used in this study were divinylbenzene based (MAbPac RP) and highly covered phenyl bonded silica (BioResolve) materials. Obviously, a higher TFA percentage would be required to maintain appropriate peak shape and resolution on other conventional silica based RP materials.

3.2. MS interpretation

RPLC-MS analyses were performed on N-deglycosylated ADC sample (no change in retention profile between the glycosylated and deglycosylated ADC samples), by using the optimised LC conditions discussed in Section 3.1. It should be noted that the mAb sequence, drug structure and expected molecular masses of the ADC variants cannot be reported due to confidentiality reasons. However, the site-specific cysteine linked ADC consists of an IgG1 mAb having two identical light chains and heavy chains held together by disulphide bonds. Each heavy chain contains an N-terminal variable domain (VH) and three constant domains (CH1, CH2, CH3), with engineered cysteines E157C in CH1 and S380C in CH3 allowing the site-specific conjugation of the drug payloads. Regarding the hydrophobicity of the drug payloads, the distribution coefficient at pH 6.8 ($\log D_{pH6.8}$) was 3.5, which is quite similar to the distribution coefficients reported for the payloads of other commercial ADCs such as brentuximab vedotin ($\log D_{pH6.8} = 3.5$) and trastuzumab emtansine ($\log D_{pH6.8} = 3.7$) [24]. For this sample, MS assignment was unambiguously obtained with a remarkable mass accuracy in the range of 20–60 ppm, which is generally expected for intact ADC analysis performed in denaturing conditions on time-of-flight (Q-TOF) instruments [2]. As shown in Fig. 3, the five peaks related to the ADC variants were all identified as drug-loaded variants, with the major peak represented by the variant loaded with 4 drugs (noted as D4). Interestingly, positional isomers were found for species loaded with 2 and 3 drugs (noted as D2, D2′, D3 and D3′, respectively). The deconvoluted mass spectra of the five peaks related to the ADC species (Fig. 3, right panel) are noted based on the number of drug payloads linked to the ADC (2–4) and clearly highlight the mass shifts related to the addition of the drug payload. In addition, fragmentation or detachment of the drug payloads was not observed under any circumstances, thus confirming the stability of the sample under the discussed analytical conditions.

4. Conclusion

In conclusion, we have demonstrated the benefits of using RPLC vs. HIC for the analysis of site-specific ADCs having relatively hydrophobic payloads. As shown, the proposed RPLC method can be useful for QC purposes for the analysis of similar site-specific ADCs, since it allows: i) to separate the unconjugated mAb from the ADC species and quantify the amount of DAR0, ii) to separate the DAR species and calculating the average DAR and DAR distribution, iii) to eventually separate positional isomers of DAR variants.

In addition, RPLC is inherently compatible with MS, thus allowing an unambiguous identification of the peaks observed on the chromatogram, thanks to the high mass accuracy of TOF-based instruments. Last but not least, mild conditions can be employed in RPLC using state-of-the-art columns (mobile phase temperature of 60 °C and 0.05%TFA/0.05%FA as mobile phase additive), to avoid potential degradation of ADC species during their analysis.

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References

[1] 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.

[2] J. Marcoux, T. Champion, O. Colas, E. Wagner-Rousset, N. Corvaïa, A. Van Dorsselaer, A. Beck, S. Cianférani, Native mass spectrometry and ion mobility characterization of trastuzumab emtansine, a lysine-linked antibody drug conjugate, Protein Sci. 24 (2015) 1210–1223.

[3] 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.
[4] B. Bobály, S. Fleury-Souverain, A. Beck, J.-L. Veuthey, D. Guillarme, S. Fekete, Current possibilities of liquid chromatography for the characterization of antibody-drug conjugates, J. Pharm. Biomed. Anal. 147 (2018) 493–505.
[5] F. Debaene, A. Bœuf, E. Wagner-Rousset, O. Colas, D. Ayoub, N. Corvaïa, A. Van Dorsselaer, A. Beck, S. Cianférani, Innovative native MS methodologies for antibody drug conjugate characterization: high resolution native MS and IM-MS for average DAR and DAR distribution assessment, Anal. Chem. 86 (2014) 10674–10683.
[6] A. Wakankar, Y. Chen, Y. Gokarn, F.S. Jacobson, Analytical methods for physicochemical characterization of antibody drug conjugates, MAbs. 3 (2011) 161–172.
[7] L.N. Le, J.M.R. Moore, J. Ouyang, X. Chen, M.D.H. Nguyen, W.J. Galush, Profiling antibody drug conjugate positional isomers: a system-of-equations approach, Anal. Chem. 84 (2012) 7479–7486.
[8] S. Fekete, J.-L. Veuthey, A. Beck, D. Guillarme, Hydrophobic interaction chromatography for the characterization of monoclonal antibodies and related products, J. Pharm. Biomed. Anal. 130 (2016) 3–18.
[9] J.R. Junutula, H. Raab, S. Clark, S. Bhakta, D.D. Leipold, S. Weir, Y. Chen, M. Simpson, S.P. Tsai, M.S. Dennis, Y. Lu, Y.G. Meng, C. Ng, J. Yang, C.C. Lee, E. Duenas, J. Gorrell, V. Katta, A. Kim, K. McDorman, K. Flagella, R. Venook, S. Ross, S.D. Spencer, W. Lee Wong, H.B. Lowman, R. Vandlen, M.X. Sliwkowski, R.H. Scheller, P. Polakis, W. Mallet, Site-specific conjugation of a cytotoxic drug to an antibody improves the therapeutic index, Nat. Biotechnol. 26 (2008) 925–932.
[10] J.P.M. Nunes, V. Vassileva, E. Robinson, M. Morais, M.E.B. Smith, R.B. Pedley, S. Caddick, J.R. Baker, V. Chudasama, Use of a next generation maleimide in combination with THIOMAB™ antibody technology delivers a highly stable, potent and near homogeneous THIOMAB™ antibody-drug conjugate (TDC), RSC Adv. 7 (2017) 24828–24832.
[11] J.D. Sadowsky, T.H. Pillow, J. Chen, F. Fan, C. He, Y. Wang, G. Yan, H. Yao, Z. Xu, S. Martin, D. Zhang, P. Chu, J. dela Cruz-Chuh, A. O'Donohue, G. Li, G. Del Rosario, J. He, L. Liu, C. Ng, D. Su, G.D. Lewis Phillips, K.R. Kozak, S.-F. Yu, K. Xu, D. Leipold, J. Wai, Development of efficient chemistry to generate site-specific disulfide-linked protein– and peptide–payload conjugates: application to THIOMAB antibody–drug conjugates, Bioconjug. Chem. 28 (2017) 2086–2098.
[12] T. Botzanowski, S. Erb, O. Hernandez-Alba, A. Ehkirch, O. Colas, E. Wagner-Rousset, D. Rabuka, A. Beck, P.M. Drake, S. Cianférani, S. Cianferani, Insights from native mass spectrometry approaches for top- and middle- level characterization of site-specific antibody-drug conjugates, MAbs 9 (2017) 801–811.
[13] S. Fekete, I. Molnár, D. Guillarme, Separation of antibody drug conjugate species by RPLC: a generic method development approach, J. Pharm. Biomed. Anal. 137 (2017) 60–69.
[14] G. Casi, D. Neri, Antibody–drug conjugates: basic concepts, examples and future perspectives, J. Control. Release 161 (2012) 422–428.
[15] P. Polakis, Antibody drug conjugates for cancer therapy, Pharmacol. Rev. 68 (2015) 3–19.
[16] M. Rodriguez-Aller, D. Guillarme, A. Beck, S. Fekete, Practical method development for the separation of monoclonal antibodies and antibody-drug-conjugate species in hydrophobic interaction chromatography, part 1: optimization of the mobile phase, J. Pharm. Biomed. Anal. 118 (2016) 393–403.
[17] A. Cusumano, D. Guillarme, A. Beck, S. Fekete, Practical method development for the separation of monoclonal antibodies and antibody-drug-conjugate species in hydrophobic interaction chromatoraphy, part 2: optimization of the phase system, J. Pharm. Biomed. Anal. 121 (2016) 161–173.
[18] B. Wiggins, L. Liu-Shin, H. Yamaguchi, G. Ratnaswamy, Characterization of cysteine-linked conjugation profiles of immunoglobulin G1 and immunoglobulin G2 antibody–drug conjugates, J. Pharm. Sci. 104 (2015) 1362–1372.
[19] T. Chen, Y. Chen, C. Stella, C.D. Medley, J.A. Gruenhagen, K. Zhang, Antibody-drug conjugate characterization by chromatographic and electrophoretic techniques, J. Chromatogr. B 1032 (2016) 39–50.
[20] A. Vailaya, C. Horváth, Retention thermodynamics in hydrophobic interaction chromatography, Ind. Eng. Chem. Res. 35 (1996) 2964–2981.
[21] A. Ehkirch, V. D'Atri, F. Rouviere, O. Hernandez-Alba, A. Goyon, O. Colas, M. Sarrut, A. Beck, D. Guillarme, S. Heinisch, S. Cianferani, An online four-dimensional HIC×SEC-IM×MS methodology for proof-of-concept characterization of antibody drug conjugates, Anal. Chem. 90 (2018) 1578–1586.
[22] S. Fekete, A. Beck, E. Wagner, K. Vuignier, D. Guillarme, Adsorption and recovery issues of recombinant monoclonal antibodies in reversed-phase liquid chromatography, J. Sep. Sci. 38 (2015) 1–8.
[23] B. Bobály, A. Beck, D. Guillarme, S. Fekete, J. Fekete, D. Guillarme, S. Fekete, Systematic evaluation of mobile phase additives for the LC–MS characterization of therapeutic proteins, Talanta 136 (2015) 60–67.
[24] A. Goyon, L. Sciascera, A. Clarke, D. Guillarme, R. Pell, Extending the limits of size exclusion chromatography: simultaneous separation of free payloads and related species from antibody drug conjugates and their aggregates, J. Chromatogr. A 1539 (2018) 19–29.