Journal of Pharmaceutical and Biomedical Analysis xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Review

Current possibilities of liquid chromatography for the characterization of antibody-drug conjugates

Balázs Bobály[a], Sandrine Fleury-Souverain[b], Alain Beck[c,1], Jean-Luc Veuthey[a], Davy Guillarme[a], Szabolcs Fekete[a,*]

[a] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CMU - Rue Michel Servet 1, 1211 Geneva 4, Switzerland
[b] Pharmacy, Geneva University Hospitals (HUG), Geneva, Switzerland
[c] Institut de Recherche Pierre Fabre, Centre d'Immunologie, 5 Avenue Napoléon III, BP 60497, 74160 Saint-Julien-en-Genevois, France

ARTICLE INFO

Article history:
Received 15 May 2017
Received in revised form 12 June 2017
Accepted 13 June 2017
Available online xxx

Keywords:
Antibody-drug-conjugate
Hydrophobic interaction chromatography
Reversed phase chromatography
Size exclusion chromatography
Multi-dimensional separation
Drug to antibody ratio

ABSTRACT

Antibody Drug Conjugates (ADCs) are innovative biopharmaceuticals gaining increasing attention over the last two decades. The concept of ADCs lead to new therapy approaches in numerous oncological indications as well in infectious diseases. Currently, around 60 CECs are in clinical trials indicating the expanding importance of this class of protein therapeutics.

ADCs show unprecedented intrinsic heterogeneity and address new quality attributes which have to be assessed. Liquid chromatography is one of the most frequently used analytical method for the characterization of ADCs. This review summarizes recent results in the chromatographic characterization of ADCs and supposed to provide a general overview on the possibilities and limitations of current approaches for the evaluation of drug load distribution, determination of average drug to antibody ratio (DAR_{av}), and for the analysis of process/storage related impurities. Hydrophobic interaction chromatography (HIC), reversed phase liquid chromatography (RPLC), size exclusion chromatography (SEC) and multidimensional separations are discussed focusing on the analysis of marketed ADCs. Fundamentals and aspects of method development are illustrated with applications for each technique. Future perspectives in hydrophilic interaction chromatography (HILIC), HIC, SEC and ion exchange chromatography (IEX) are also discussed.

© 2017 Elsevier B.V. All rights reserved.

Contents

1. Introduction 00
2. Hydrophobic interaction chromatography (HIC) 00
3. Reversed phase liquid chromatography 00
4. Size-exclusion chromatography 00
5. Multidimensional separation 00
6. Determination of drug load distribution and average DAR 00
7. Future perspectives 00
   7.1. HILIC 00
   7.2. HIC 00
   7.3. SEC 00
   7.4. IEX 00
8. Conclusion 00
   Acknowledgement 00
   References 00

1. Introduction

Antibody Drug Conjugates (ADCs) are innovative biopharmaceuticals gaining more and more attention over the last two decades. They consist of potent small molecular cytotoxic drugs,

* Corresponding author.
E-mail address: szabolcs.fekete@unige.ch (S. Fekete).
[1] www.cipf.com

http://dx.doi.org/10.1016/j.jpba.2017.06.022
0731-7085/© 2017 Elsevier B.V. All rights reserved.

which are covalently bound to a recombinant monoclonal antibody (mAb), *via* synthetic linkers. The antibody part has a key role in selective target cell recognition, while the conjugated potent cytotoxic drug is responsible for the effective elimination of the target cells [1,2]. This concept lead to new therapy approaches in numerous oncological [3–5] and infectious indications [6,7] as well as for arthrosclerosis [8], anti-inflammatory treatments [9] and immunosuppression [10]. Currently, more than 60 CECs are in clinical trials and this number is expected to increase in the future [11–13].

The first generation ADC, namely gemtuzumab ozogamicin (Mylotarg®) was approved by the US Food and Drug Administration (FDA) in 2000. The acid-labile hydrazon linker of gemtuzumab ozogamicin was not stable enough in the circulatory system, which resulted in non-specific drug release and limited clinical benefits. Finally, the drug has been voluntarily withdrawn from the market in 2010. Second generation ADCs, brentuximab vedotin (Adcetris®) and trastuzumab emtansine (Kadcyla®) were commercialzed in 2011 and 2013, respectively. These are currently the only two ADCs approved by the FDA and European Medicines Agency (EMA). Brentuximab vedotin is a cysteine conjugated ADC, in which monomethyl auristatin E (MMAE) warheads are attached to a chimeric anti-CD30 IgG1 mAb via cathepsin cleavable linkers. In cysteine conjugated ADCs, reduced inter-chain disulfides are conjugated, which finally results in drug load distribution of 0–8 for IgG1 and 0–12 for IgG2 host mAbs. Trastuzumab emtansine is a lysine conjugated ADC which consists of a humanized anti-HER2 IgG1 and emtansine (DM-1) payloads conjugated via non-cleavable linkers. Lysine is a prevalent amino acid present in mAb sequences. From 90 possible conjugation sites of trastuzumab, around 20 are highly solvent accessible and capable of conjugation. The resulting mixture of conjugated species is unprecedentedly heterogeneous and shows drug load distribution from 0 to 8. The conjugation process of second generation ADCs provides IgGs with an average drug to antibody ratio (DAR_{av}) of 4.0 for brentuximab vedotin and 3.5 for trastuzumab emtansine. Conjugation reaction has to be well controlled, since DAR_{av} and drug load distribution influence efficacy, pharmacokinetics and stability of the final product. The desired DAR_{av} for second generation ADCs is around 4. Lower DAR may not efficiently eliminate target cells, while higher DARs possess shorter half-life in circulatory system and tend to form aggregates more easily [4]. Third generation ADCs are based on engineered mAbs possessing conjugation sites at defined positions, enabling the production of more homogeneous pools of ADC payloads. Currently, more than 40 site specific conjugation technologies are available. Conjugation site engineering is often combined with alternative conjugation chemistries. At least 10 of third generation ADCs have reached clinical development yet [3].

Heterogeneity of ADCs may be due to each component of the product. Among variations related to the host mAb itself (i.e. amino acid clipping, chemical modifications, glycosylation, aggregation, etc.) and to its production process/storage (i.e. particulates, leachables, endotoxins, sterility, etc.), several other critical quality attributes (CQAs) that are more ADC specific have also to be assessed. These include DAR, drug distribution, conjugated impurities, free drug species and residual conjugation solvents [14]. Analytical techniques used for the characterization of mAbs [15,16] can usually be also used for the analysis of ADCs [17–21]. Several review papers recently appeared on ADC characterization, involving the use of mass spectrometry [19,20] and separation based techniques [17,18], showing their key role of these analytical methodologies in this field. Here, we wanted to exclusively focus on liquid chromatographic approaches for the analytical characterization of ADCs. Due to the limited number of marketed products, this review focuses mainly on second generation ADCs, namely brentuximab vedotin and trastuzumab emtansine. The inherent heterogeneity of the lysine conjugated trastuzumab emtansine significantly limits the chromatographic applications for its characterization. The discussion of the cysteine conjugated ADC brentuximab vedotin is more detailed.

The aim of this review is to provide a general overview on the possibilities and limitations of liquid chromatography for ADC characterization, to help practicing chromatographers in developing reliable separation methods. Basic theoretical concepts are illustrated, together with most recent results in chromatographic method development and applications. The review discusses hydrophobic interaction chromatography (HIC) for the characterization of drug load distribution (which measures the homogeneity of the ADC population) and DAR_{av} under native conditions, reversed phase chromatography (or reverse phase, RPLC) for the determination of DAR_{av} and process/storage related impurities, using denaturing conditions and finally, size exclusion chromatography (SEC) for the determination of ADC size variants (above all aggregates). Multidimensional chromatographic solutions were also critically discussed, and future perspectives in method development, particle technology and possible separation methods are provided.

2. Hydrophobic interaction chromatography (HIC)

HIC can be considered as a historical chromatographic technique for the purification and characterization of proteins. HIC separates sample components according to their relative hydrophobicity using mild conditions (e.g. physiological pH conditions, ambient mobile phase temperature and no or moderated amount of organic solvents) which help to preserve the native-like conformation of protein species. This is the main benefit of HIC compared to RPLC in which proteins are separated under harsh, denaturing conditions. In HIC, non-covalent protein complexes, like cysteine conjugated ADCs preserve their conformation and do not dissociate into subunits like in RPLC. HIC separates individually loaded variants, while maintaining their Y-shaped mAb-structure and thus enabling the evaluation of drug-load distribution and the calculation of DAR_{av}. On the other hand, HIC is not sufficiently efficient to separate positional isomers at the protein level, and due to the high mobile phase salt concentration, MS hyphenation is rarely feasible.

Recent review papers detailed HIC method development and also showed some useful applications [22–24]. The goal of the present review was to summarize the main points of these works and complete them with most recent results. HIC starts with an injection of the samples into a buffered high-salt concentration mobile phase, which leads to the adsorption of proteins at the surface of a moderately apolar stationary phase. Due to the complex nature of protein surface interactions, the adsorption process is still not well understood. It may consist of several solute – mobile phase – stationary phase interactions [22,25]. Elution starts with decreasing salt concentration (inverse salt gradient). The eluting mobile phase is a low ionic strength (10–50 mM) buffer containing few percent of organic modifier, such as isopropanol, ethanol, etc. to facilitate desorption and enhance recovery. Modern HIC stationary phases are made of porous as well as non-porous silica as well as polymer particles, which are either bonded with short alkyl (ethyl-hexyl) chains or modified by ether or alkylamide chemistries. The ligand density is low compared to RPLC stationary phases, which makes the surface of HIC particles less hydrophobic. Various column dimensions are available for analytical scale separations from 4.6 mm × 3.5 mm to 4.6 mm × 250 mm packed with particles of 2.5–10 μm. It is worth mentioning that not all HIC materials adapted for mAbs will appropriately work for highly hydrophobic multiply conjugated DAR species [26]. In some cases, the high DAR species cannot be eluted from the most hydrophobic materials [26]. Upon selection of the stationary phase, salt type

Please cite this article in press as: B. Bobály, et al., Current possibilities of liquid chromatography for the characterization of antibody-drug conjugates, J. Pharm. Biomed. Anal. (2017), http://dx.doi.org/10.1016/j.jpba.2017.06.022

Download English Version:

https://daneshyari.com/en/article/7627905

Download Persian Version:

https://daneshyari.com/article/7627905

Daneshyari.com