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# Bioanalytical Assays for Pharmacokinetic and Biodistribution Study of Antibody-Drug Conjugates

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## ABSTRACT

**Antibody-drug conjugates (ADCs) are produced by the chemical linkage of cytotoxic agents and monoclonal antibodies. The complexity and heterogeneity of ADCs and the low concentration of cytotoxic agent released in vivo poses big challenges to their bioanalysis. Understanding the pharmacokinetic behavior, exposure-safety, and exposure-efficacy relationships of ADCs is needed for their successful development. Accurate analytical methods are required to evaluate intact ADCs, total antibody, released small molecule cytotoxins, and related metabolites. The selection of appropriate bioanalysis methods for comprehensive analysis of ADCs is mainly dependent on the properties of cytotoxic agents, the chemical linker, and the attachment sites. The quality of the information about the whole pharmacokinetic profile of ADCs has been improved due to the development and improvement of analytical strategies for detection of ADCs, such as ligand-binding assays and mass spectrometry-related techniques. In this article, we will focus on the bioanalytical assays that have been used in the pharmacokinetic study of ADCs and discuss their advantages, current limitations, and potential challenges.**

## SIGNIFICANCE STATEMENT

**This article describes bioanalysis methods which have been used in pharmacokinetic study of ADCs and discusses the advantages, disadvantages and potential challenges of these assays. This review is useful and helpful and will provide insights and reference for bioanalysis and development of ADCs.**

## Introduction

Antibody-drug conjugates (ADCs) are an innovative and significant kind of complex biopharmaceuticals with highly cytotoxic small molecular compounds (payloads) covalently bound to monoclonal antibodies through a chemical linker (McCombs and Owen, 2015; Parslow et al., 2016; Zhang et al., 2022). The typical chemical structure of an ADC is shown in Fig. 1A. Compared with monoclonal antibodies, the chemical structures of ADCs are more complex and heterogeneous. The objective of development of ADCs is the specific and targeted delivery of small molecular cytotoxic payload to cancer cells. Meanwhile, they are less exposed in normal tissues, which results in improving the therapeutic index of anti-tumor treatment and minimizing the corresponding toxic side effects (Abdollahpour-Alitappeh et al., 2019; Hafeez et al., 2020; Marei et al., 2022; Tarantino et al., 2022).

Generally, the chemical structure of ADCs has three parts: monoclonal antibody, chemical linker, and small molecular cytotoxic payload (Dal Corso et al., 2017; Bargh et al., 2019; Ashman et al., 2023). ADCs specifically combine with the highly expressed proteins (target antigen) on the surface of cancer cells through monoclonal antibody to generate ADC antigen complex, and then enter the cells through the endocytosis mediated by clathrin; thereafter, the drug carrier enters the tumor cells to release drug and kill tumor cells. The metabolism and action mechanism of ADC is shown in Fig. 1, B and C. The antibody part of ADCs should be able to selectively combine with the antigen molecules that are specifically expressed or overexpressed on the surface of cancer cells (McCombs and Owen, 2015; Yao et al., 2016). The cytotoxicity of the payload of ADC should be very high so that the payload can kill tumor cells at low concentrations reached in the cells after the ADC is

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**ABBREVIATIONS:** ADC, antibody-drug conjugate; CIQA, cryoimaging quantitative autoradiography; DAR, drug-to-antibody ratio; DM1, mertansine; FMT, fluorescence molecular tomography; LBA, ligand-binding assay; LC–MS/MS, liquid chromatography tandem mass spectrometry; LSC, liquid scintillation counter; MS, mass spectrometry; PET, positron emission tomography; SPE, solid phase extraction; SPECT, single photon emission computed tomography; T-DM1, trastuzumab emtansine.

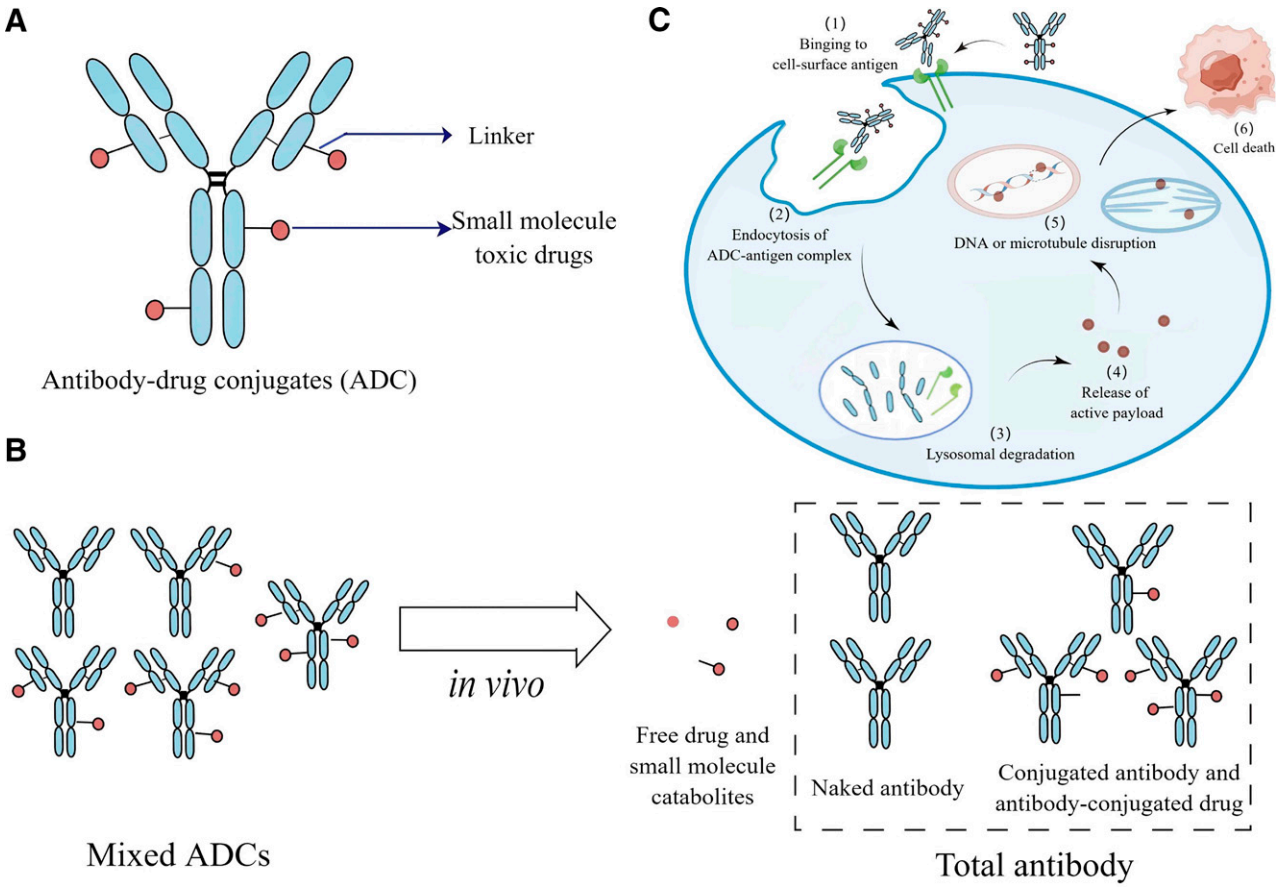


**Fig. 1.** (A) Typical chemical structure of ADC. (B) Metabolism of ADC in vivo. (C) Action mechanism of ADC.

distributed to solid tumor tissue (McCombs and Owen, 2015). The number of cytotoxic agents connected with the antibody molecule is limited; therefore, the biophysical and pharmacokinetic characteristics of ADCs are not seriously affected contrast to the corresponding monoclonal antibodies. ADCs are heterogeneous mixtures which have different drug-to-antibody ratios (DARs). The DAR distribution of ADCs is dynamically changing in vivo. Furthermore, the linkers of ADCs should be stable enough in systemic circulation to ensure that the payloads remain connected to the antibodies, but once the ADCs distribute into solid tumor tissue and enter the cancer cells, the linkers should be broken easily and completely to release the small molecular cytotoxic payload to kill the cancer cells (Cazzamalli et al., 2017; Dal Corso et al., 2017; Deonarain and Yahioglu, 2021; Nicolaou et al., 2021; Teicher and Morris, 2022; Ashman et al., 2023).

ADC is a hot track of innovative drugs at present, and its indications are mainly solid tumors (Joubert et al., 2020; Chia, 2022). So far, there are 310 ADC drugs with known targets in the world. Most of these drugs are in the early clinical stage. Only 15 of them have been successfully marketed and approved by the U.S. Food and Drug Administration for cancer treatment in clinical applications (summarized in Table 1), 13 candidate drugs have entered the clinical stage III, and 156 and 137 drugs are in the preclinical stage and clinical stage I. Understanding the pharmacokinetic behavior of ADCs is important for their development, optimization, and clinical application (Han and Zhao, 2014; Kraynov et al., 2016; Zhang et al., 2019). The pharmacokinetic behaviors of ADCs are summarized and shown in Table 2. Accurate analytical methods for pharmacokinetic study of ADCs should monitor different kinds of analytes, such as intact antibody drug conjugates, conjugated small-molecular toxins, total antibodies, and free small-molecular toxins and their related metabolites (Dere et al., 2013). The selection of appropriate analytical methods for comprehensive analysis of ADCs is mainly associated with the properties of the chemical linker, small molecular cytotoxic agents, and the attachment sites (Wakankar et al., 2011; Källsten et al., 2018; Cahuzac and Devel, 2020; Pretto and FitzGerald, 2021). At present, label-free bioanalytical assays and labeled bioanalytical methods are the two main types of bioanalytical assays for determination of ADCs-derived compounds in plasma or tissues. Label-free bioanalytical assays mainly refer to ligand-binding assays (LBAs), mass spectrometry (MS)-based methods, or the combination of the above assays (Wang et al., 2016; Todoroki et al., 2018; Lechner et al., 2019; Chang and Shah, 2020; Todoroki et al., 2020; Zhang et al., 2023). In most cases of labeled ADCs, a radioactive isotope or a fluorescent reporter should be incorporated within the structure of the payload and/or the monoclonal antibody. The in vivo fate of ADCs can be monitored by noninvasive imaging assays just like positron emission tomography (PET), single photon emission computed tomography (SPECT) and fluorescence molecular tomography (FMT) (Ilovich et al., 2015; Giddabasappa et al., 2016; Brand et al., 2018; Carmon and Azhdarinia, 2018).

The aim of this article is to outline the bioanalytical methods that have been used in pharmacokinetic study of ADCs and discuss their associated merits, current limitations and potential challenges. We believe this review is helpful and will provide insights and reference for bioanalysis and development of ADCs.

TABLE 1

ADCs approved by the Food and Drug Administration

| ADC | Target Antigen | Payload | Linker | Average DAR | Indication | FDA Approval |
|---|---|---|---|---|---|---|
| Mylotarg | CD33 | Calicheamicins | Cleavable | 2-3 | Acute myeloid leukemia | 2000 |
| Adcetris | CD30 | Monomethy lauristatin E (MMAE) | Cleavable | 4 | Hodgkin's lymphoma | 2011 |
| Kadcyla | HER2 | Mertansine (DM1) | Noncleavable | 3.5 | Breast cancer | 2013 |
| Besponsa | CD22 | Calicheamicins | Cleavable | 5–7 | B-cell precursor acute lymphoblastic leukemia | 2017 |
| Lumoxiti | CD22 | Pseudomonas aeruginosa exotoxin (PE38) | Cleavable | / | Hyperleukocytic acute leukemia | 2018 |
| Polivy | CD79b | Monomethy lauristatin E (MMAE) | Cleavable | 3.5 | Non-Hodgkin's lymphoma | 2019 |
| Euhertu | HER2 | Exatecan derivative (Dxd) | Cleavable | 8 | Breast cancer, gastric cancer | 2019 |
| Padcev | Nectin-4 | Monomethy lauristatin E (MMAE) | Cleavable | 4 | Urothelial carcinoma | 2019 |
| Trodelvy | TROP2 | SN38 (7-Ethyl-10-hydroxycamptothecin) | Cleavable | 8 | Triple negative breast cancer | 2020 |
| Blenrep | BCMA | Mono Methylauristatin F (MMAF) | Cleavable | 4 | Multiple myeloma | 2020 |
| Akalux | EGFR | IRDye700DX | Noncleavable | / | Head and neck cancer | 2020 |
| Zynlonta | CD19 | PBD | Cleavable | 2.3 | Non-Hodgkin's lymphoma | 2021 |
| Disitamab Vedotin For Iicction | HER2 | Monomethy lauristatin E (MMAE) | Cleavable | 3.5 | Gastric cancer | 2021 |
| Tivdak | TF | Monomethy lauristatin E (MMAE) | Cleavable | 4 | Cervical carcinoma | 2021 |
| Elahere | FR$\alpha$ | DM4 | Noncleavable | 3.4 | Ovarian cancer | 2022 |

### Analysis of ADCs by Ligand-Binding Assays

The LBAs are generally used for detection of biologic large molecular compounds (Thway, 2016). The critical procedure of LBAs is the equilibrium reaction between the ligand and the protein or antibody that is directed against the target analytes. The final time point of the reaction reveals the amounts of the monitored analytes in different biologic samples. The analytes in biologic samples can be captured and detected by most LBAs with antibodies as critical reagents (Jani et al., 2016). ELISAs are the gold standard of LBAs for detection of different analytes in various biologic simples (Fischer et al., 2015). Monoclonal antibodies and polyclonal antibodies are the preferred critical reagents in LBAs. To generate monoclonal antibodies or polyclonal antibodies, rabbits, goats, and sheep are usually used as the host species, which are immunized with therapeutic proteins and adjuvant (Lipman et al., 2005; Thway, 2016).

The chemical structure of ADCs is very complicated. Bioanalysis and pharmacokinetic study of ADCs is a difficult task. The efficacy and toxicity of ADCs are associated with antibody conjugated drug, free drug, and total antibody, which should be monitored simultaneously. For analysis of ADCs, liquid chromatography tandem mass spectrometry (LC-MS/MS) is usually used to detect payload with small molecular weight and LBAs are usually used to detect total antibody or conjugated antibody whose molecular weight is large. For example, Stephan et al. developed bioanalysis assay based on the ELISA technique for determination of MC-MMAF conjugates and anti-CD22-MCC-DM1 in mouse plasma. For analysis of the total anti-CD22 antibodies, the antibodies were captured by CD22 extracellular domain (ECD) and detected by a goat anti-human IgG Fc horseradish peroxidase-conjugated antibody GxhFc-horseradish peroxidase. Moreover, for analysis of the drug-conjugated anti-CD22 antibody, recombinant CD22 extracellular domain and anti-mertansine (DM1) or anti-MMAF biotinylated antibody were used as the capture reagent and detection reagent, respectively (Stephan et al., 2008). Lewis Phillips and his collaborators developed ELISA-based analytical method for determination of trastuzumab-maytansinoid conjugates (HER2 breast cancer treatment drug) in mouse plasma. For detection of trastuzumab-maytansinoid, HER2 extracellular domain and goat anti-human Fc integrated horseradish peroxidase-conjugated were used as the capture reagent and detection reagent, respectively (Lewis Phillips et al., 2008). Generally speaking, the advantages of LBAs include low cost, high throughput, easy implementation, and high sensitivity for determination of large molecules. The disadvantages of LBAs are summarized as follows (Mou et al., 2018): (1) LBAs could not measure the DAR of ADCs; (2) the sensitivity of LBAs is limited for detecting the metabolites of ADCs; (3) the selectivity

TABLE 2

Pharmacokinetic characteristics of ADCs

| | ADCs |
|---|---|
| Molecular weight (Da) | 150K |
| Administration route | Intravenous administration |
| Distribution | The apparent distribution volume of ADCs is generally small, close to plasma volume, and limited tissue distribution; The apparent distribution volume of payload is relatively large, and it is easier to enter the tissue; May be the substrate of transporter |
| Metabolism | Phase I and II metabolism, proteolysis. |
| Excretion | ADCs and antibodies mainly circulate in the body; The main excretory pathway for payload and its metabolites are bile and renal excretion. |
| Half-life time | The half-life time of both ADC and free small-molecular toxins are relatively longer. |
| Pharmacokinetic linearity | Generally, they are linear at high dose, but nonlinear at low dose. |
| Target analytes | Antibody drug conjugates, total antibodies, conjugated small-molecular toxins, free small-molecular toxins and their related metabolites |
| Bioanalysis method | Ligand binding assay, LC-MS/MS, CE-MS/MS, etc. |
| Immunogenicity | Yes |
| Heterogeneity | Mixture |

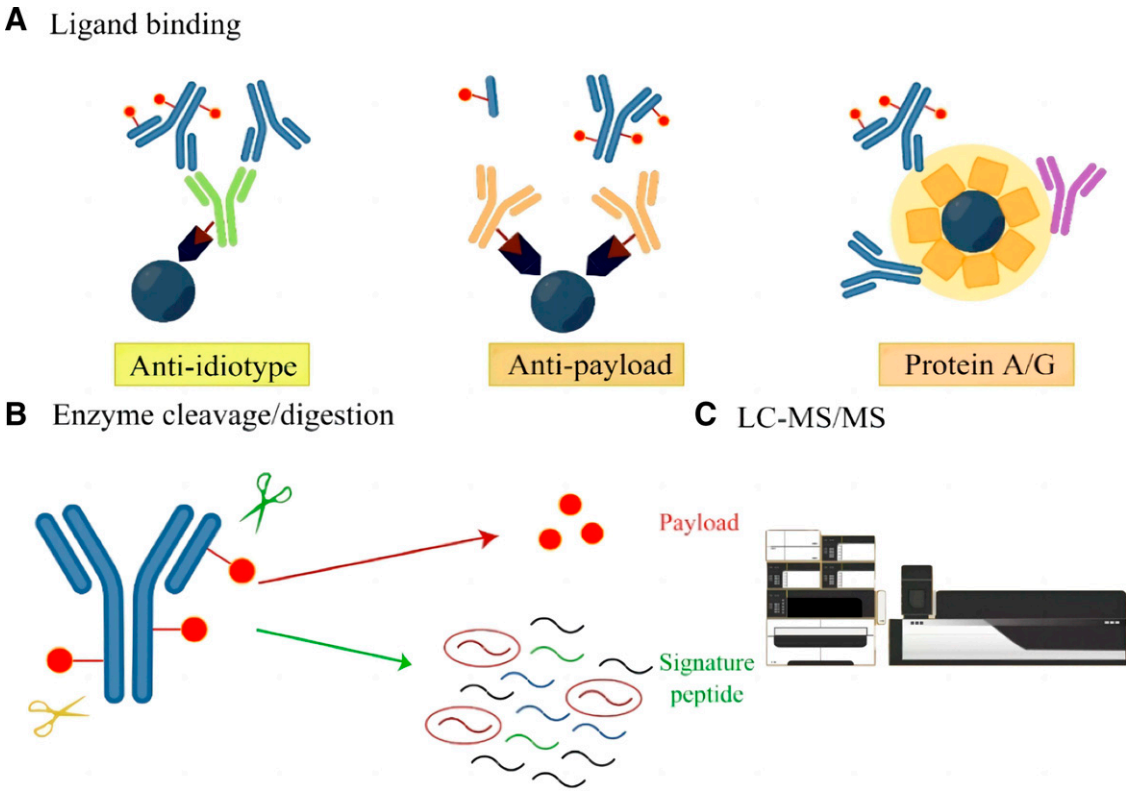


**Fig. 2.** The typical procedures of hybrid LBA-LC–MS/MS assays: (A) ligand binding, (B) enzyme cleavage/digestion; (C) LC–MS/MS analysis.

of LBAs with antibodies to capture and detect the analytes is not high; (4) LBAs cannot provide the comprehensive information about chemical structure and sequence of ADCs; (5) the antibodies used in LBAs have potential cross-reactivity; (6) the multiplexing capability of LBAs is limited; and (7) the time for development of antibodies of LBAs is long.

### Analysis of ADCs by Hybrid LBA-LC-MS/MS Assays

Hybrid LBA-LC-MS/MS assays are complementary alternatives to LBAs for bioanalysis of ADCs. The typical procedures for bioanalysis of conjugated-payload of ADCs by hybrid LBA and LC–MS/MS methods are as follows: first, the conjugated-payload of ADCs are captured by corresponding reagents, such as generic capture reagents, anti-payload, or anti-idiotype capture reagents; second, after elution, the conjugated-payload of ADCs are digested with trypsin or cathepsin-B enzyme; and third, the generated peptides from enzyme digestion of the conjugated-payload of ADCs are detected by LC-MS/MS with MRM transitions (Qu et al., 2017; Wei et al., 2018; Zhu et al., 2020; Panda et al., 2022). To avoid interference from endogenous human IgGs, unique peptides from variable regions are usually selected for detection of ADCs. Anti-human Fc antibodies could be selected as the immunoaffinity capture reagent to capture and purify the targeted antibodies in biologic matrix, which leads to the improved specificity and sensitivity of the hybrid LBA-LC-MS/MS assay (Ouyang et al., 2012; Furlong et al., 2014). Fig. 2 illustrates the typical procedures of hybrid LBA/LC-MS/MS assay (Wang et al., 2016; Zhu et al., 2020). For example, Liu et al. developed a hybrid LBA and LC-MS/MS assay to determine Bristol-Myers Squibb ADC in monkey plasma. The selective capture reagent used in their study was a unique monoclonal antibody. The reagents were biotinylated with EZ-Link Sulfo-NHS-LC-Biotins, and the corresponding analytes in biologic matrix were immunocaptured. Samples were slowly loaded onto the kit. The captured analytes were eluted from the cartridges using ammonium bicarbonate and then digested with cathepsin B enzyme. After that, the generated peptides of the conjugated payload can be analyzed by LC-MS/MS method (Liu et al., 2015). Moreover, a hybrid LBA and LC-MS/MS assay was developed by Faria et al. to quantify total ADC and total antibody of MEDI4276 in human plasma. In their study, anti-idiotype antibodies were used to capture MEDI4276 followed by enzyme digestion with trypsin. The released payloads were used to measure total ADC and the produced peptide from enzyme digestion of the complementary determining region of the corresponding ADC was selected to determine total antibodies (Faria et al., 2019). Generally, hybrid LBA-LC-MS/MS methods have numerous advantages such as high sensitivity, high selectivity and multiplex capability. Furthermore, the methods can also offer valuable structure information of ADCs, such as DAR and drug load. Moreover, hybrid LBA-LC-MS/MS assays also have some disadvantages, for example, high cost, complicated data interpretation, complicated instrument operation, lower throughput, and low sensitivity for detection of intact ADC.

### Analysis of Payload of ADCs by MS Methods

The payloads of ADCs are the small molecule toxins that are not linked to the antibody. The small molecule toxins can be released from the catabolism of ADCs in vivo. LC–MS/MS assay is an excellent technique to detect payloads of ADCs due to its high selectivity and high sensitivity. Solid phase extraction (SPE) and protein precipitation with organic solvents are usually selected as the sample preparation assay to remove proteins in biologic matrix before chromatographic separation. The lower limit of quantification of the LC–MS/MS assay should be much lower because the concentration of the released drug is much low in biologic samples. For instance, Olivier Heudi et al. developed and validated a specific and sensitive LC–MS/MS assay coupled with online SPE technology to determine maytansinoid in human serum. The free

thiol moiety of DM1 was first reduced by the chemical reagent [tris (2-carboxyethyl) phosphine] and then blocked with another agent N-ethylmaleimide. After that, the samples were diluted before the online SPE on a $C_{18}$ cartridge. The LC–MS/MS assay coupled with on-line SPE was successfully used for the determination of DM1 in human serum (Heudi et al., 2016). Liu et al. developed and validated a convenient LC–MS/MS assay for simultaneous quantification of three catabolites (DM1, MCC-DM1, Lys-MCC-DM1) of the ADC trastuzumab emtansine (T-DM1) in vitro cellular samples. Protein precipitation was selected as the sample processing method in this study. The developed assay showed excellent linearity (1–100 nM) with an $r^2$ value more than 0.995 (Liu et al., 2017). Generally speaking, the MS method for determination of payloads of ADC has numerous advantages, such as high selectivity, high sensitivity, and multiplex capability. The MS method also has some drawbacks; for example, the MS method may be affected by the non-volatile salts and endogenous compounds in biologic samples (Redman et al., 2016; Qu et al., 2017).

### Analysis of ADCs by Labeled Bioanalytical Methods

The pharmacokinetic of ADCs in vivo can be also studied with radiolabeled techniques (Shadid et al., 2017). Radiolabeled ADCs can be used as the typical model for their pharmacokinetic study. The payload or antibody component of ADCs can be labeled with radioisotope. Then the in vivo fate of ADCs can be investigated by monitoring the radioisotopes through the use of imaging techniques, such as PET, SPECT, and FMT, which are sensitive and noninvasive (Chia et al., 2020; Chomet et al., 2020; Pellico et al., 2021). The temporal window for imaging can be dictated by the half-life of the labeled radioisotope. The biodistribution of ADCs could be revealed by the monitoring of the radiometals (Marciscano and Thorek, 2018). For example, Winnik et al. studied the biodistribution of [$^{89}$Zr]Zr-Desferrioxamine*-T-DM1 in Balb/c and NOD/SCID mice by Micro PET/CT image technique (Fig. 3A). The results of their study indicated that uptake (dose/g) of [$^{89}$Zr]Zr-DFO*-T-DM1 was 5.0 ± 1.8% (Cho et al., 2020). Sijbrandi et al. studied the pharmacokinetic behaviors of $^{89}$Zr-trastuzumab, $^{89}$Zr-trastuzumab-Mal-AF and $^{89}$Zr-trastuzumab-Lx-AF in NCI-N87–bearing mice by PET-CT image technology. The results of their study suggested that the pharmacokinetic and tissue distribution properties of these three ADCs are similar. The concentrations of these three ADCs are much higher in tumor tissues compared with the normal tissues which means that the ADCs have excellent tumor targeting (Sijbrandi et al., 2017).

Lutje et al. used SPECT to investigate the biodistribution of $^{111}$In-labeled ADCs in BALB/c nude mice (Lutje et al., 2018). The distribution of different $^{111}$In-labeled ADCs in tumor tissues could be visualized clearly at 3 days after subcutaneous injection. Boswell et al. studied the tumor to heart ratio of $^{111}$In-labeled anti-tomoregulin monomethyl auristatin E in male C.B-17 SCID beige mice by SPECT-CT imaging technology (Fig. 3B). The tumor-to-heart ratio decreased along with the increasement of the dose of ADC (Boswell et al., 2012).

Gupta et al. investigated the distribution and tumor targeting potential of an anti-IL13R$\alpha_2$ ADC in A375 xenograft-bearing mice by FMT imaging technique (Gupta et al., 2021). FMT revealed an excellent distribution profile for anti-IL13R$\alpha_2$ ADC. The tested ADC showed a dose associated anti-tumor effect on A375 xenograft-bearing mice. When the dose is 3 mg/kg, the proportion of complete responders is 90%. Giddabasappa et al. studied the biodistribution and tumor targeting of Anti-5T4 ADC in female nu/nu tumor bearing mice by FMT imaging technology (Giddabasappa et al., 2016). The protocol for assessing the distribution and tumor targeting of biologic drugs by FMT imaging technique is shown in Fig. 3C. Their study proved that FMT is a useful technique to reveal the pharmacokinetic behavior of ADCs in vivo.

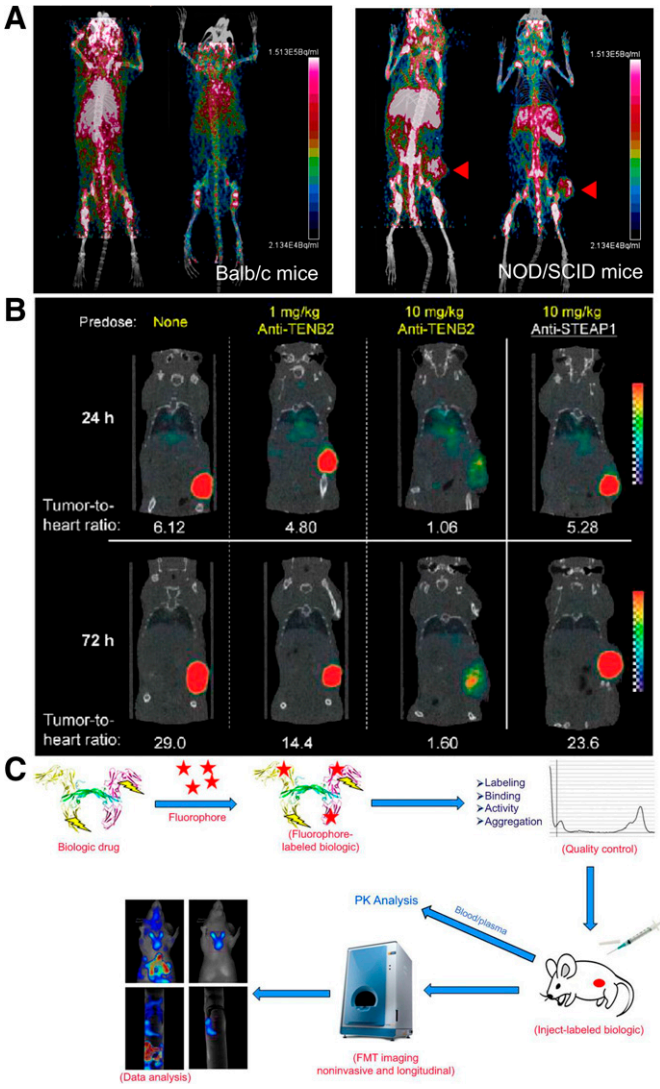


**Fig. 3.** (A) Biodistribution study of [$^{89}$Zr]Zr-DFO-T-DM1 and [$^{89}$Zr]Zr-DFO-T-DM1 in non-tumor bearing Balb/c mice and NOD/SCID mice with s.c HER2-overexpressing SK-OV-3 human ovarian cancer xenografts (arrow) by microPET/CT. Reprinted with permission from Cho et al., Nuclear Medicine and Biology 2020, 84–85:11–19. Copyright © 2019 Elsevier Inc. All rights reserved. (B) Biodistribution study of $^{111}$In-anti-TENB2-MMAE (3 mg/kg) at 24 hours or 72 hours after injection in mice by SPECT/CT. Reprinted with permission from Boswell et al., Journal of Nuclear Medicine 2012, 53(9):1454–1461. Copyright © 2018 SNMMI. (C) Schematic representing the stepwise protocol followed in evaluating biodistribution and targeting of biologic drugs using FMT imaging. Ab/ADC (biologic drug) was labeled with VT680 by NHS ester reaction. After labeling, the VT680-conjugated Ab/ADCs were qualitatively and/or quantitatively evaluated for VT680 labeling, stability, binding to the antigen, and cytotoxic activity by in vitro methods. After these quality control evaluations, in vivo biodistribution and tumor targeting were determined longitudinally by FMT imaging and PK analysis of blood and tissues. Reprinted with permission from Giddabasappa et al., Mol Cancer Ther 2016, 15:2530–2540. Copyright © 2016, American Association for Cancer Research.

Dual radiolabeling of different components of ADCs is also a good option which can be used to study the in vivo behavior of ADCs (Alley et al., 2009). For example, Joey A. Muns et al. studied the biodistribution and blood kinetics of a dual radiolabeled trastuzumab-[$^{195m}$Pt]Lx-DFO-$^{89}$Zr by immuno-PET imaging technique (Muns et al., 2018). As indicated via $^{195m}$Pt/$^{89}$Zr dual labeling, the results of their study showed that the tested ADC trastuzumab-[$^{195m}$Pt] Lx-DFO-$^{89}$Zr is stable in blood and its tumor targeting is good. Ilovich et al. used dual-isotope cryo-imaging quantitative autoradiography (CIQA) imaging technique

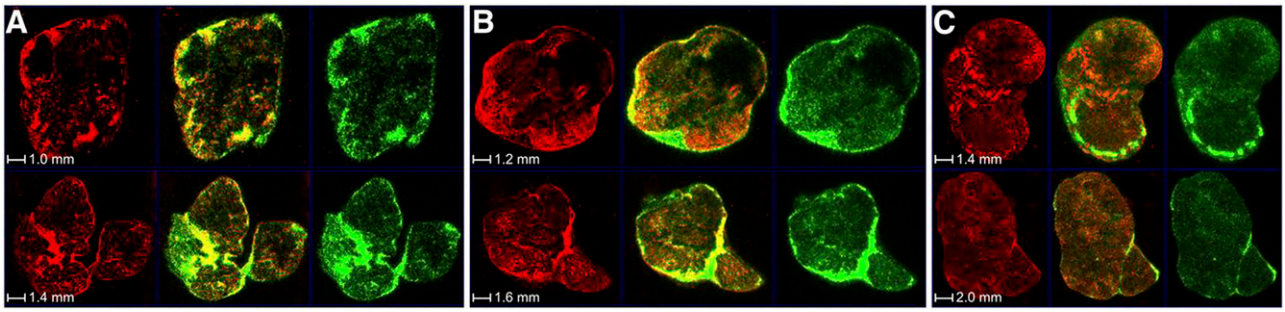


**Fig. 4.** Representative sections of HEK-293 GCC2 tumors (top) and HEK-293 tumors (bottom) excised at 1 hour (A), 24 hours (B), and 96 hours (C) after tracer injection. Voxels are 0.025, 0.025, and 0.025 mm. H signal is red, $^{111}$In signal is green, and both signals coregistered is yellow. Strikingly, image of HEK-293 GCC2 at 24 hours shows initial diffusion of drug away from antibody accumulation site and deeper into tumor. Reprinted with permission from Ilovich et al., J Nucl Med 2018, 59:1461–1466. Copyright © 2018 SNMMI.

to investigate the biodistribution and payload delivery of an ADC which has $^{111}$In-labeled antibody and $^{3}$H-labeled payload MMAE (Ilovich et al., 2018). In their study, CIQA clearly showed the amount of the released payload in blood and tissues at different time points (Fig. 4). Chari et al. investigated the biodistribution of an ADC SGN-75 in tumor-bearing mice which has [$^{14}$C] labeled MMAF and [$^{3}$H] labeled anti-CD70 antibody (Chari et al., 2014). The accumulation of $^{14}$C from MMAF or $^{3}$H from antibody in tumor and various normal tissues could be detected by CIQA. They found that MMAF-derived radioactivity accumulated preferentially in tumor tissues. Cahuzac et al. used dual radiolabeling and ex vivo digital imaging technique to monitor the in vivo fate of an ADC (Fig. 5) which was dual-labeled with $^{3}$H and $^{14}$C (Cahuzac et al., 2022). These studies confirmed the feasibility of dual radiolabeling for pharmacokinetic study of ADCs. Moreover, liquid scintillation counter (LSC) is a radioactivity meter that uses a liquid scintillator to accept radiation and convert it into fluorescent photons. It is a useful tool for pharmacokinetics study of ADCs (Kamath and Iyer, 2016). Total radiation and radioactivity can be quantified from biologic samples by LSC. For example, Okeley et al. studied the cellular kinetics and uptake of $^{14}$C-labeled SGN-35 by LSC (Okeley et al., 2010). Girish et al. investigated the pharmacokinetic behavior of [$^{3}$H]-labeled Trastuzumab Emtansine (T-DM1) in rats by LSC. The total radioactivity in rat plasma, bile, tissues, urine, and feces are determined by the LSC (Shen et al., 2012). Bolleddula et al. studied the catabolism and pharmacokinetic of [$^{3}$H] labeled TAK-164 in tumor-bearing mice by LSC (Bolleddula et al., 2020). The results of their study showed that the terminal half-life of the [$^{3}$H]-labeled TAK-164 is 41 hours in mice

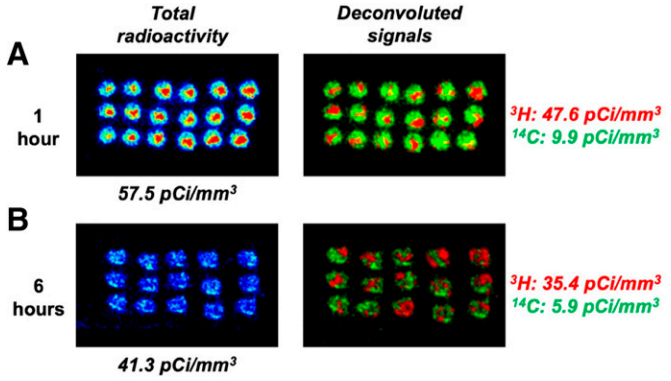


**Fig. 5.** Ex vivo dual radio-imaging of 4T1 tumor sections enables the quantification of both components of [$^{3}$H/$^{14}$C]-2 1 hour (A) or 6 hours (B) after administration ($n$ = 3 mice per time). Reprinted with permission from Cahuzac et al., J Med Chem 2022, 656953–6968. Copyright © 2022 American Chemical Society.

plasma, and its clearance is 0.75 mL/h per kilogram. However, LSC also has some limitations. For instance, chemical, color, or physical quenching will happen during sample processing which result in reducing the counting efficiency and thus the quenching correction should be performed for LSC detection. Moreover, for LSC, the energy used for separation of $\alpha$ and $\beta$ particles is depended on many factors. Therefore, in the process of measuring the total $\alpha$ and $\beta$ radioactivity in LSC, the correct setting of different parameters is very important. In addition, the energy released by the anions and particles in the solution can also affect the detection process of LSC (Stapleton, 2022).

Generally, labeled analytical methods for bioanalysis of ADC have some advantages, such as high sensitivity, visualization, and being noninvasive. However, the selectivity of labeled analytical assays is not high. Labeled analytical assays cannot distinguish the ADCs and their metabolites when they all have the radioactive element. Furthermore, the radioactive labeled element may cause immunoreactivity and change the in vivo behavior of ADCs.

## Current Challenges and Future Perspectives

ADCs are a novel and unique kind of anticancer therapeutic. Due to the complexity and heterogeneity of ADCs and their dynamic changes in vivo, the pharmacokinetic profile of ADCs is correspondingly complex, which poses big challenges for the bioanalysis of ADCs. Bioanalysis of ADCs should simultaneously focus on intact ADCs, total antibody, released small molecule cytotoxins, and related metabolites. During these years, analytical methods requiring a prelabeling of ADCs (such as PET and PMT) and label-free analytical methods (such as LBA and MS based assays) have been widely used for pharmacokinetic study of ADCs. The future development of bioanalysis technologies, especially the application of new mass spectrometry-based techniques, can support more accurate and comprehensive analytical tools for monitoring the in vivo fate of ADCs. Improvements of chromatographic technologies can provide high resolution separations for ADCs derived analytes. The combination of different analytical technologies is leading to a better and a more thorough understanding of the pharmacokinetic behavior, structure-exposure-safety/efficacy relationships of ADCs, which is helpful and important for the designing and development of ADCs.

### Data Availability

The authors declare that all the data supporting the findings of this study are contained within the paper.

### Authorship Contributions

*Wrote or contributed to the writing of the manuscript:* Yin, Xu, Zhao, Gu.

## References


Abdollahpour-Alitappeh M, Lotfinia M, Gharibi T, Mardaneh J, Farhadihosseinabadi B, Larki P, Faghfourian B, Sepehr KS, Abbaszadeh-Goudarzi K, Abbaszadeh-Goudarzi G et al. (2019) Antibody-drug conjugates (ADCs) for cancer therapy: Strategies, challenges, and successes. *J Cell Physiol* **234:**5628–5642.

Alley SC, Zhang X, Okeley NM, Anderson M, Law CL, Senter PD, and Benjamin DR (2009) The pharmacologic basis for antibody-auristatin conjugate activity. *J Pharmacol Exp Ther* **330:**932–938.

Ashman N, Bargh JD, Walsh SJ, Greenwood RD, Tiberghien A, Carroll JS, and Spring DR (2023) Peroxide-cleavable linkers for antibody-drug conjugates. *Chem Commun (Camb)* **59:**1841–1844.

Bargh JD, Isidro-Llobet A, Parker JS, and Spring DR (2019) Cleavable linkers in antibody-drug conjugates. *Chem Soc Rev* **48:**4361–4374.

Bolleddula J, Shah A, Shadid M, Kamali A, Smith MD, and Chowdhury SK (2020) Pharmacokinetics and Catabolism of [$^3$H]TAK-164, a Guanylyl Cyclase C Targeted Antibody-Drug Conjugate. *Drug Metab Dispos* **48:**1239–1245.

Boswell CA, Mundo EE, Zhang C, Stainton SL, Yu SF, Lacap JA, Mao W, Kozak KR, Fourie A, Polakis P et al. (2012) Differential effects of predosing on tumor and tissue uptake of an 111In-labeled anti-TENB2 antibody-drug conjugate. *J Nucl Med* **53:**1454–1461.

Brand C, Sadique A, Houghton JL, Gangangari K, Ponte JF, Lewis JS, Pillarsetty NVK, Konner JA, and Reiner T (2018) Leveraging PET to image folate receptor α therapy of an antibody-drug conjugate. *EJNMMI Res* **8:**87.

Cahuzac H and Devel L (2020) Analytical Methods for the Detection and Quantification of ADCs in Biological Matrices. *Pharmaceuticals (Basel)* **13:**462.

Cahuzac H, Sallustrau A, Malgorn C, Beau F, Barbe P, Babin V, Dubois S, Palazzolo A, Thai R, Correia I et al. (2022) Monitoring In Vivo Performances of Protein-Drug Conjugates Using Site-Selective Dual Radiolabeling and Ex Vivo Digital Imaging. *J Med Chem* **65:**6953–6968.

Carmon KS and Azhdarinia A (2018) Application of Immuno-PET in Antibody-Drug Conjugate Development. *Mol Imaging* **17:**1536012118801223.

Cazzamalli S, Corso AD, and Neri D (2017) Linker stability influences the anti-tumor activity of acetazolamide-drug conjugates for the therapy of renal cell carcinoma. *J Control Release* **246:**39–45.

Chang HP and Shah DK (2020) Determination of ADC Concentration by Ligand-Binding Assays. *Methods Mol Biol* **2078:**361–369.

Chari RV, Miller ML, and Widdison WC (2014) Antibody-drug conjugates: an emerging concept in cancer therapy. *Angew Chem Int Ed Engl* **53:**3796–3827.

Chia CSB (2022) A Patent Review on FDA-Approved Antibody-Drug Conjugates, Their Linkers and Drug Payloads. *ChemMedChem* **17:**e202200032.

Chia PL, Parakh S, Tsao MS, Pham NA, Gan HK, Cao D, Burvenich IJG, Rigopoulos A, Reilly EB, John T et al. (2020) Targeting and Efficacy of Novel mAb806-Antibody-Drug Conjugates in Malignant Mesothelioma. *Pharmaceuticals (Basel)* **13:**289.

Cho H, Al-Saden N, Lam H, Möbus J, Reilly RM, and Winnik MA (2020) A comparison of DFO and DFO* conjugated to trastuzumab-DM1 for complexing $^{89}$Zr - In vitro stability and in vivo microPET/CT imaging studies in NOD/SCID mice with HER2-positive SK-OV-3 human ovarian cancer xenografts. *Nucl Med Biol* **84–85:**11–19.

Chomet M, Schreurs M, Nguyen M, Howng B, Villanueva R, Krimm M, Vasiljeva O, van Dongen GAMS, and Vugts DJ (2020) The tumor targeting performance of anti-CD166 Probody drug conjugate CX-2009 and its parental derivatives as monitored by $^{89}$Zr-immuno-PET in xenograft bearing mice. *Theranostics* **10:**5815–5828.

Dal Corso A, Cazzamalli S, Gébleux R, Mattarella M, and Neri D (2017) Protease-Cleavable Linkers Modulate the Anticancer Activity of Noninternalizing Antibody-Drug Conjugates. *Bioconjug Chem* **28:**1826–1833.

Deonarain MP and Yahioglu G (2021) Current strategies for the discovery and bioconjugation of smaller, targetable drug conjugates tailored for solid tumor therapy. *Expert Opin Drug Discov* **16:**613–624.

Dere R, Yi JH, Lei C, Saad OM, Huang C, Li Y, Baudys J, and Kaur S (2013) PK assays for antibody-drug conjugates: case study with ado-trastuzumab emtansine. *Bioanalysis* **5:**1025–1040.

Faria M, Peay M, Lam B, Ma E, Yuan M, Waldron M, Mylott Jr WR, Liang M, and Rosenbaum AI (2019) Multiplex LC-MS/MS Assays for Clinical Bioanalysis of MEDI4276, an Antibody-Drug Conjugate of Tubulysin Analogue Attached via Cleavable Linker to a Biparatopic Humanized Antibody against HER-2. *Antibodies (Basel)* **8:**11.

Fischer SK, Joyce A, Spengler M, Yang T-Y, Zhuang Y, Fjording MS, and Mikulskis A (2015) Emerging technologies to increase ligand binding assay sensitivity. *AAPS J* **17:**93–101.

Furlong MT, Titsch C, Xu W, Jiang H, Jemal M, and Zeng J (2014) An exploratory universal LC-MS/MS assay for bioanalysis of hinge region-stabilized human IgG4 mAbs in clinical studies. *Bioanalysis* **6:**1747–1758.

Giddabasappa A, Gupta VR, Norberg R, Gupta P, Spilker ME, Wentland J, Rago B, Eswaraka J, Leal M, and Sapra P (2016) Biodistribution and Targeting of Anti-5T4 Antibody-Drug Conjugate Using Fluorescence Molecular Tomography. *Mol Cancer Ther* **15:**2530–2540.

Gupta P, Jiang ZK, Yang B, Manzuk L, Rosfjord E, Yao J, Lemon L, Noorbehesht K, David J, Puthenveetil S et al. (2021) Targeting and pharmacology of an anti-IL13Rα2 antibody and antibody-drug conjugate in a melanoma xenograft model. *MAbs* **13:**1958662.

Hafeez U, Parakh S, Gan HK, and Scott AM (2020) Antibody-Drug Conjugates for Cancer Therapy. *Molecules* **25:**4764.

Han TH and Zhao B (2014) Absorption, distribution, metabolism, and excretion considerations for the development of antibody-drug conjugates. *Drug Metab Dispos* **42:**1914–1920.

Heudi O, Barteau S, Picard F, and Kretz O (2016) Quantitative analysis of maytansinoid (DM1) in human serum by on-line solid phase extraction coupled with liquid chromatography tandem mass spectrometry - Method validation and its application to clinical samples. *J Pharm Biomed Anal* **120:**322–332.

Ilovich O, Natarajan A, Hori S, Sathirachinda A, Kimura R, Srinivasan A, Gebauer M, Kruip J, Focken I, Lange C et al. (2015) Development and Validation of an Immuno-PET Tracer as a Companion Diagnostic Agent for Antibody-Drug Conjugate Therapy to Target the CA6 Epitope. *Radiology* **276:**191–198.

Ilovich O, Qutaish M, Hesterman JY, Orcutt K, Hoppin J, Polyak I, Seaman M, Abu-Yousif AO, Cvet D, and Bradley DP (2018) Dual-Isotope Cryoimaging Quantitative Autoradiography: Investigating Antibody-Drug Conjugate Distribution and Payload Delivery Through Imaging. *J Nucl Med* **59:**1461–1466.

Jani D, Allinson J, Berisha F, Cowan KJ, Devanarayan V, Gleason C, Jeromin A, Keller S, Khan MU, Nowatzke B et al. (2016) Recommendations for Use and Fit-for-Purpose Validation of Biomarker Multiplex Ligand Binding Assays in Drug Development. *AAPS J* **18:**1–14.

Joubert N, Beck A, Dumontet C, and Denevault-Sabourin C (2020) Antibody-Drug Conjugates: The Last Decade. *Pharmaceuticals (Basel)* **13:**245.

Källsten M, Hartmann R, Artemenko K, Lind SB, Lehmann F, and Bergquist J (2018) Qualitative analysis of antibody-drug conjugates (ADCs): an experimental comparison of analytical techniques of cysteine-linked ADCs. *Analyst (Lond)* **143:**5487–5496.

Kamath AV and Iyer S (2016) Challenges and advances in the assessment of the disposition of antibody-drug conjugates. *Biopharm Drug Dispos* **37:**66–74.

Kraynov E, Kamath AV, Walles M, Tarcsa E, Deslandes A, Iyer RA, Datta-Mannan A, Sriraman P, Bairlein M, Yang JJ et al. (2016) Current Approaches for Absorption, Distribution, Metabolism, and Excretion Characterization of Antibody-Drug Conjugates: An Industry White Paper. *Drug Metab Dispos* **44:**617–623.

Lechner A, Giorgetti J, Gahoual R, Beck A, Leize-Wagner E, and François YN (2019) Insights from capillary electrophoresis approaches for characterization of monoclonal antibodies and antibody drug conjugates in the period 2016-2018. *J Chromatogr B Analyt Technol Biomed Life Sci* **1122–1123:**1–17.

Lewis Phillips GD, Li G, Dugger DL, Crocker LM, Parsons KL, Mai E, Blättler WA, Lambert JM, Chari RV, Lutz RJ et al. (2008) Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. *Cancer Res* **68:**9280–9290.

Lipman NS, Jackson LR, Trudel LJ, and Weis-Garcia F (2005) Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources. *ILAR J* **46:**258–268.

Liu A, Kozhich A, Passmore D, Gu H, Wong R, Zambito F, Rangan VS, Myler H, Aubry AF, Arnold ME et al. (2015) Quantitative bioanalysis of antibody-conjugated payload in monkey plasma using a hybrid immuno-capture LC-MS/MS approach: Assay development, validation, and a case study. *J Chromatogr B Analyt Technol Biomed Life Sci* **1002:**54–62.

Liu Y, Zhou F, Sang H, Ye H, Chen Q, Yao L, Ni P, Wang G, and Zhang J (2017) LC-MS/MS method for the simultaneous determination of Lys-MCC-DM1, MCC-DM1 and DM1 as potential intracellular catabolites of the antibody-drug conjugate trastuzumab emtansine (T-DM1). *J Pharm Biomed Anal* **137:**170–177.

Lütje S, Gerrits D, Molkenboer-Kuenen JD, Herrmann K, Fracasso G, Colombatti M, Boerman OC, and Heskamp S (2018) Characterization of Site-Specifically Conjugated Monomethyl Auristatin E- and Duocarmycin-Based Anti-PSMA Antibody-Drug Conjugates for Treatment of PSMA-Expressing Tumors. *J Nucl Med* **59:**494–501.

Marciscano AE and Thorek DLJ (2018) Role of noninvasive molecular imaging in determining response. *Adv Radiat Oncol* **3:**534–547.

Marei HE, Cenciarelli C, and Hasan A (2022) Potential of antibody-drug conjugates (ADCs) for cancer therapy. *Cancer Cell Int* **22:**255.

McCombs JR and Owen SC (2015) Antibody drug conjugates: design and selection of linker, payload and conjugation chemistry. *AAPS J* **17:**339–351.

Mou S, Huang Y, and Rosenbaum AI (2018) ADME Considerations and Bioanalytical Strategies for Pharmacokinetic Assessments of Antibody-Drug Conjugates. *Antibodies (Basel)* **7:**41.

Muns JA, Montserrat V, Houthoff HJ, Codée-van der Schilden K, Zwaagstra O, Sijbrandi NJ, Merkul E, and van Dongen GAMS (2018) In Vivo Characterization of Platinum(II)-Based Linker Technology for the Development of Antibody-Drug Conjugates: Taking Advantage of Dual Labeling with $^{195m}$Pt and $^{89}$Zr. *J Nucl Med* **59:**1146–1151.

Nicolaou KC, Pan S, Pulukuri KK, Ye Q, Rigol S, Erande RD, Vourloumis D, Nocek BP, Munneke S, Lyssikatos J et al. (2021) Design, Synthesis, and Biological Evaluation of Tubulysin Analogues, Linker-Drugs, and Antibody-Drug Conjugates, Insights into Structure-Activity Relationships, and Tubulysin-Tubulin Binding Derived from X-ray Crystallographic Analysis. *J Org Chem* **86:**3377–3421.

Okeley NM, Miyamoto JB, Zhang X, Sanderson RJ, Benjamin DR, Sievers EL, Senter PD, and Alley SC (2010) Intracellular activation of SGN-35, a potent anti-CD30 antibody-drug conjugate. *Clin Cancer Res* **16:**888–897.

Ouyang Z, Furlong MT, Wu S, Sleczka B, Tamura J, Wang H, Suchard S, Suri A, Olah T, Tymiak A et al. (2012) Pellet digestion: a simple and efficient sample preparation technique for LC-MS/MS quantification of large therapeutic proteins in plasma. *Bioanalysis* **4:**17–28.

Panda D, Dash BP, Manickam S, and Boczkaj G (2022) Recent advancements in LC-MS based analysis of biotoxins: Present and future challenges. *Mass Spectrom Rev* **41:**766–803.

Parslow AC, Parakh S, Lee FT, Gan HK, and Scott AM (2016) Antibody-Drug Conjugates for Cancer Therapy. *Biomedicines* **4:**4.

Pellico J, Gawne PJ, and T M de Rosales R (2021) Radiolabelling of nanomaterials for medical imaging and therapy. *Chem Soc Rev* **50:**3355–3423.

Pretto F and FitzGerald RE (2021) In vivo safety testing of Antibody Drug Conjugates. *Regul Toxicol Pharmacol* **122:**104890.

Qu M, An B, Shen S, Zhang M, Shen X, Duan X, Balthasar JP, and Qu J (2017) Qualitative and quantitative characterization of protein biotherapeutics with liquid chromatography mass spectrometry. *Mass Spectrom Rev* **36:**734–754.

Redman EA, Mellors JS, Starkey JA, and Ramsey JM (2016) Characterization of Intact Antibody Drug Conjugate Variants Using Microfluidic Capillary Electrophoresis-Mass Spectrometry. *Anal Chem* **88:**2220–2226.

Shadid M, Bowlin S, and Bolleddula J (2017) Catabolism of antibody drug conjugates and characterization methods. *Bioorg Med Chem* **25:**2933–2945.

Shen BQ, Bumbaca D, Saad O, Yue Q, Pastuskovas CV, Khojasteh SC, Tibbitts J, Kaur S, Wang B, Chu YW et al. (2012) Catabolic fate and pharmacokinetic characterization of trastuzumab emtansine (T-DM1): an emphasis on preclinical and clinical catabolism. *Curr Drug Metab* **13:**901–910.

Sijbrandi NJ, Merkul E, Muns JA, Waalboer DC, Adamzek K, Bolijn M, Montserrat V, Somsen GW, Haselberg R, Steverink PJ et al. (2017) A Novel Platinum(II)-Based Bifunctional ADC Linker Benchmarked Using 89Zr-Desferal and Auristatin F-Conjugated Trastuzumab. *Cancer Res* **77:**257–267.

Stapleton A-M (2022) Liquid scintillation counting, in: Radioactive Sample Counting: Principles and Practice (Second Edition): IPEM report 85, IOP Publishing, Bristol, United Kingdom.

Stephan JP, Chan P, Lee C, Nelson C, Elliott JM, Bechtel C, Raab H, Xie D, Akutagawa J, Baudys J et al. (2008) Anti-CD22-MCC-DM1 and MC-MMAF conjugates: impact of assay format on pharmacokinetic parameters determination. *Bioconjug Chem* **19:**1673–1683.

Tarantino P, Carmagnani Pestana R, Corti C, Modi S, Bardia A, Tolaney SM, Cortes J, Soria JC, and Curigliano G (2022) Antibody-drug conjugates: Smart chemotherapy delivery across tumor histologies. *CA Cancer J Clin* **72:**165–182.

Teicher BA and Morris J (2022) Antibody-drug Conjugate Targets, Drugs, and Linkers. *Curr Cancer Drug Targets* **22:**463–529.

Thway TM (2016) Fundamentals of large-molecule protein therapeutic bioanalysis using ligand-binding assays. *Bioanalysis* **8:**11–17.
Todoroki K, Mizuno H, Sugiyama E, and Toyo'oka T (2020) Bioanalytical methods for therapeutic monoclonal antibodies and antibody-drug conjugates: A review of recent advances and future perspectives. *J Pharm Biomed Anal* **179:**112991.
Todoroki K, Yamada T, Mizuno H, and Toyo'oka T (2018) Current Mass Spectrometric Tools for the Bioanalyses of Therapeutic Monoclonal Antibodies and Antibody-Drug Conjugates. *Anal Sci* **34:** 397–406.
Wakankar A, Chen Y, Gokarn Y, and Jacobson FS (2011) Analytical methods for physicochemical characterization of antibody drug conjugates. *MAbs* **3:**161–172.
Wang J, Gu H, Liu A, Kozhich A, Rangan V, Myler H, Luo L, Wong R, Sun H, Wang B et al. (2016) Antibody-drug conjugate bioanalysis using LB-LC-MS/MS hybrid assays: strategies, methodology and correlation to ligand-binding assays. *Bioanalysis* **8:**1383–1401.
Wei C, Su D, Wang J, Jian W, and Zhang D (2018) LC–MS Challenges in Characterizing and Quantifying Monoclonal Antibodies (mAb) and Antibody-Drug Conjugates (ADC) in Biological Samples. *Curr Pharmacol Rep* **4:**45–63.
Yao H, Jiang F, Lu A, and Zhang G (2016) Methods to Design and Synthesize Antibody-Drug Conjugates (ADCs). *Int J Mol Sci* **17:**194.
Zhang D, Dragovich PS, Yu SF, Ma Y, Pillow TH, Sadowsky JD, Su D, Wang W, Polson A, Khojasteh SC et al. (2019) Exposure-Efficacy Analysis of Antibody-Drug Conjugates Delivering an Excessive Level of Payload to Tissues. *Drug Metab Dispos* **47:**1146–1155.
Zhang X, Kwok T, Zhou M, Du M, Li V, Bo T, Huang T, and Chen T (2023) Imaged capillary isoelectric focusing (icIEF) tandem high resolution mass spectrometry for charged heterogeneity of protein drugs in biopharmaceutical discovery. *J Pharm Biomed Anal* **224:**115178.
Zhang X, Zhang Q, Xie L, An J, Wang S, Yu X, and Zhao X (2022) The Value of Whole-Tumor Texture Analysis of ADC in Predicting the Early Recurrence of Locally Advanced Cervical Squamous Cell Cancer Treated With Concurrent Chemoradiotherapy. *Front Oncol* **12:**852308.
Zhu X, Huo S, Xue C, An B, and Qu J (2020) Current LC-MS-based strategies for characterization and quantification of antibody-drug conjugates. *J Pharm Anal* **10:**209–220.

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