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Review

Comparison of bioanalytical methods for the quantitation of PEGylated human insulin

Kristine de Dios *, Anthony Manibusan, Robin Marsden, Jason Pinkstaff

Ambrx, Inc., 10975 North Torrey Pines Road, La Jolla, CA 92037, USA

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ABSTRACT

Purpose: The quality of bioanalytical data is dependent upon selective, sensitive, and reproducible analytical methods. With evolving technologies available, bioanalytical scientists must assess which is most appropriate for their molecule through proper method validation. For an early stage PEGylated insulin program, the characteristics of four platforms, ELISA, ECL, Gyrolab, and LC–MS/MS, were evaluated using fit-for-purpose method development and validation, while also evaluating costs.

Method: Methods selected for validation required acceptable performance based on satisfaction of a priori criteria prior to proceeding to subsequent stages of validation. LBA pre-validation included reagent selection, evaluation of matrix interference, and range determination. LC–MS/MS pre-validation included selection of a signature peptide; optimization of sample preparation, HPLC, and LC–MS/MS conditions; and calibration range determination. Pre-study validation tested accuracy and precision (mean bias criteria ± 30%; precision ≤ 30%). Pharmacokinetic (PK) parameters were estimated for an in vivo study with WinNonlin noncompartmental analysis. Statistics were performed with JMP using ANOVA and Tukey–Kramer post hoc analysis. A cost analysis was performed for a 200-sample PK study using the methods from this study.

Results: All platforms, except Gyrolab, were taken through validation. However, a typical Gyrolab method was included for the cost analysis. Ranges for the ELISA, ECLA, and LC–MS/MS were 8.52–75, 2.09–125, and 100–1000 ng/mL, respectively, and accuracy and precision fell within a priori criteria. PK samples were analyzed in the 3 validated methods. PK profiles and parameters are similar for all methods, except LC–MS/MS, which differed at t = 24 h and with AUC0-24. Further investigation into this difference is warranted. The cost analysis identified the Gyrolab platform as the most expensive and ELISA as the least expensive, with method specific consumables attributing significantly to costs.

Conclusions: ECLA had a larger dynamic range and sensitivity, allowing accurate assessment of PK parameters. Although this method was more expensive than the ELISA, it was the most appropriate for the early stage PEGylated insulin program. While this case study is specific to PEGylated human insulin, it highlights the importance of evaluating and selecting the most appropriate platform for bioanalysis during drug development.

Abbreviations: ACN, acetonitrile; AUC, area under the curve; C_{max}, maximal plasma concentration; %C.V., percent coefficient of variation; DTT, dithiothreitol; ECL, electrochemiluminescence; ECLA, electrochemiluminescence assay; ELISA, enzyme-linked immunosorbent assay; LC–MS/MS, liquid chromatography mass spectrometry; FA, formic acid; HPLC, high performance liquid chromatography; HQC, high quality control; IPA, isopropyl alcohol; IS, internal standard; LBA, ligand-binding assay; LLOQ, lower limit of quantitation; LQC, low quality control; mAb, monoclonal antibody; MeOH, methanol; MQC, mid quality control; PEG, polyethylene glycol; PK, pharmacokinetic; QC, quality control; %R.E., percent recovery efficiencies; RLU, relative light units; SD, Sprague-Dawley; STZ, streptozotocin; %T.E., percent total error; TFA, tri-fluoro acetic acid; ULOQ, upper limit of quantitation.

* Corresponding author. Tel.: +1 858 875 2421.
E-mail address: kristine.dedios@ambrx.com (K. de Dios).

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Contents

1. Introduction

The quality of bioanalytical data derived from preclinical and clinical studies is completely dependent upon analytical methods that are selective, sensitive, and reproducible (FDA, 2001). With evolving technologies and platforms available for bioanalysis, and the increasing number of macromolecules in development, bioanalytical scientists must accurately assess which method is most appropriate for their molecule. The various methods and platforms available should be evaluated through proper method development and validation, each informed by guidance documents.

Several techniques are available for the bioanalysis of macromolecules, but ligand binding assays (LBAs) are at the forefront. The most common LBA platform is the ELISA (Myler et al., 2011), requiring two binding reagents, one as a capture and the other as a detection reagent. The ELISA is a simple, relatively inexpensive, well-understood and established method. However, it is plagued with inadequate sensitivity and dynamic range, as well as large sample and reagent volume requirements. The electrochemiluminescent assay (ECLA) platform provides several advantages over ELISA, including a wider dynamic range, improved sensitivity, and reduced volume requirements. The disadvantages of ECLA include the cost of plates and the problem of a single source supplier (Kahn and Findlay, 2010). A nanoscale LBA, the Gyrolab, utilizes microfluidic technology to perform an immunoassay on a compact disc (CD) with micro-columns and structures containing an inlet, volume-definition chambers, and an overflow channel. Roman et al. (2011) and Mora et al. (2010) found that this platform offers sensitivity and dynamic range equivalent to the ECLA while reducing analyst hands-on time. But they also noted that key issues with this platform included the potential for carryover, the inability to perform runs in parallel, and the cost of the instrument and consumables.

Liquid chromatography (LC) used in tandem with mass spectrometry (MS/MS) was predominantly used for the bioanalysis of small molecules due to well-defined analyte structure and metabolites (Viswanathan et al., 2007). Recent advances in instrumentation and techniques have expanded the use of LC–MS/MS to macromolecule analysis (Ezan et al., 2009; Heudi et al., 2008). LC–MS/MS methods may be able to achieve similar sensitivity to that of an immunoassay with improved specificity and reduced method development time. In comparison to immunoassays, LC–MS/MS methods for large molecule quantitation require specialized equipment and extensive sample manipulations, and they are highly complex and fairly expensive, thus hampering their widespread application (Ezan et al., 2009).

Several publications have compared bioanalytical platforms with regard to assay performance (Ellis et al., 2012; Guglielmo-Viret et al., 2005; Heudi et al., 2008; Mora et al., 2010; Roman et al., 2011) including pharmacokinetic (PK) analysis (Mora et al., 2010; Roman et al., 2011), but a comprehensive evaluation of multiple platforms has not yet been published. We evaluated the characteristics of four platforms, ELISA, ECL, Gyrolab, and LC–MS/MS, using fit-for-purpose method development and validation, while also evaluating the costs associated with each platform for an early stage PEGylated insulin (PEG-insulin) program.

2. Materials and methods

2.1. Common reagents

Site-specifically PEGylated recombinant human insulin (PEG-insulin) was prepared as previously described (Miao et