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Applications of a planar electrochemiluminescence platform to support regulated studies of macromolecules: Benefits and limitations in assay range☆

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ABSTRACT

Development and validation of ligand binding methods that can measure therapeutic antibodies (TA) accurately and precisely are essential for bioanalysis that supports regulated pharmacokinetic (PK) and toxicokinetic (TK) studies. Non-bead (planar) electrochemiluminescence (ECL) methods are known to have high sensitivity and a wide assay range and are therefore potentially useful in supporting research studies in the early phases of development as well as for diagnostic fields and multiplex biomarker applications. Here, we demonstrate the applications for using ECL for regulated studies associated with protein drug development. Three planar ECL methods were developed, validated, and implemented to quantify three different TAs to support PK/TK studies. An automated liquid handler was used for the preparation of standards, quality controls, and validation samples to minimize assay variability. Robustness and ruggedness were tested during pre-study validations.

During method optimization, the potential assay ranges were 3 log orders. To improve assay accuracy and precision, assay ranges in all 3 methods were truncated by at least 50% at the upper end before proceeding to pre-study validations. All 3 methods had assay ranges of about 2 logs during pre-study validations. The inter-assay accuracy and precision during pre-study validations were <6% and 8%, respectively. The total error of the assays was <15% for both in-study and pre-study validations in all 3 methods.

With the incorporation of a robotic workstation we concluded that performance in all 3 planar ECL methods was extremely precise and accurate during pre-study and in-study validations, enabling >90% assay success during sample analyses. Although there were limitations in the assay ranges, the strength of this technology in assay accuracy, precision, and reproducibility can be beneficial for macromolecule analyses in support of PK and TK studies in a regulated environment.

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1. Introduction

In drug development, conventional enzyme-linked immunosorbent assays (ELISAs) are commonly used for the quantification of macromolecules to support pharmacokinetic (PK) and toxicokinetic (TK) studies. These assays usually have a working range limited to about 2 logs, while concentrations of biological samples from PK and TK typically span 3 to 5 orders of magnitude. Samples collected at the C_{max} timepoints from animals dosed with high concentrations of therapeutic antibody (TA) often need to be diluted at least a thousand fold to be in the working range for conventional ELISAs. Each additional dilution step introduces a compounding error that affects data quality for proper PK assessment. In addition, higher dilution may artificially shift the binding equilibrium of the TA with the soluble target protein ligand, which in turn may introduce uncertainty in the quantification of the unbound "free" TA. A desirable method, therefore, would be one that is sensitive, has a wide dynamic range, and has a minimal need for sample dilution for bioanalytical efficiency. In addition, the method should also demonstrate sufficient accuracy and precision during pre-study and in-study validations and should be robust enough to support regulated preclinical and clinical studies [1,2].

New technologies beyond conventional ELISAs have evolved. Among ligand binding assay (LBA) platforms, divergent analytical technologies such as chemiluminescence and electrochemiluminescence (ECL) are available; whereas the platform differs from manufacturer to manufacturer. A few chemiluminescence-based ELISA assays were developed, validated, and implemented in research, diagnostic, and clinical study support [3–5]. Planar (non-bead) ECL methods have been used in research at

☆ Pre- and in-study validation of LBAs using Meso Scale 6000 for PK assessments.

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Table 1
Summary of reagents and buffers used in each method.

Method	A	B	C
Capture reagent	His tagged target protein, 4.0 μg/mL	Anti-idiotypic mouse monoclonal antibody (Clone 1), 2.0 μg/mL	Anti-idiotypic mouse monoclonal antibody (Clone Y), 2.0 μg/mL
Pretreatment	1:20	1:100	1:400
Assay/blocking buffer	1 M NaCl in I-Block	1% BSA, 1 M NaCl, 0.5% Tween 20 in 1× PBS	1% BSA, 1 M NaCl, 0.5% Tween 20 in 1× PBS
Detection reagent	Biotinylated anti-idiotypic mouse monoclonal antibody (1.0 μg/mL)-followed by ruthenium-labeled streptavidin (1.0 μg/mL)	Ruthenium labeled anti-idiotypic mouse monoclonal antibody (Clone 2), (0.5 μg/mL)	Ruthenium labeled anti-idiotypic mouse monoclonal antibody (Clone Z), (0.5 μg/mL)
Tripropylamine buffer	1:4	1:8	1:8

the early development phase, for multiplex biomarkers, and for immunogenicity testing where the methodology was semi- or quasi-quantitative [6–7]. So far, other bead-based ECL methods have been developed to support PK assessments of TAs for research studies [8–9] and clinical studies.

To adopt a new technology such as the ECL method in a regulated environment, it is necessary to validate the hardware and software that were used for data acquisition and interface in addition to the validation of the LBA method. Only a few technology companies offer hardware and software that are compliant with 21CFR Part 11 regulation. Lack of 21CFR Part 11-compliant software could impose undesirable process modifications in interfacing the raw data to the laboratory information management system LIMS systems for sample management and data regression. We followed the installation and operation qualification in adopting the planar ECL technology MSD® for the intended use of supporting PK and TK studies to assure regulatory compliance.

To have a robust method with sufficient accuracy and precision, each method was developed and validated following the processes summarized in Fig. 1. Method development included feasibility, optimization, and qualification; method validation included pre-study and in-study validations. The pre-study validation process conformed to the FDA guidance for supporting PK and TK studies as well as to the recommendations of LBA method validation described in a position paper [1–2,10]. The method validation included demonstrations of accuracy, precision, robustness, reproducibility, selectivity, and specificity, as well as analyte stability under the various storage conditions that the samples could be subjected to. Random error (measured by the imprecision of the method) is the major contributor to assay variation resulting in pre-study validation failure. Combinations of systemic and random errors exceeding the FDA guidelines [1] could lead to unsolicited investigation for plausible root causes during pre-study and/or in-study validations. The goal of the current study was to develop and validate methods that have both a wide dynamic range and that also have sufficient accuracy and precision using planar ECL technology. In this paper, we present the performance validation of 3 planar ECL-based bioanalytical methods to quantify 3 different TAs in either cynomolgus monkey or rat serum to support regulated PK/TK studies. The use of an automated liquid handler was incorporated into the methods during pre-study and in-study validations to minimize assay variation.

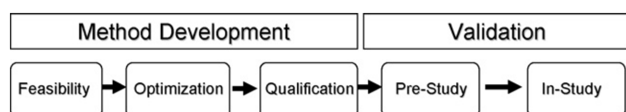

Fig. 1. Overview of method development and validation activities.

2. Experimental

2.1. Reagents

Standard MSD 96-well microplates and tripropylamine read buffer (4× MSD read buffer T) were from Meso Scale Discovery ("MSD"; Gaithersburg, MD). Standard MSD® 96-well microplates and tripropylamine read buffer (4× MSD® read buffer T) were from MSD® (Gaithersburg, MD, USA). The following reagents were from Amgen Inc. (Thousand Oaks, CA, USA): TAs-A, -B, and -C; capture reagents, target protein of the TA-A tagged with histidine, and anti-idiotypic mouse monoclonal antibodies (clone 1 against TA-B and clone Y against TA-C); detection systems of biotin-conjugated anti-idiotypic mouse monoclonal antibody (clone A against TA-A), ruthenium-labeled anti-idiotypic mouse monoclonal antibody (clone 2 against TA-B), ruthenium-labeled anti-idiotypic mouse monoclonal antibody (clone Z against TA-C), and ruthenium-labeled streptavidin (Sulfo-TAG Streptavidin). Standards (STD), validation samples (VS), and quality controls (QC) were prepared by spiking the TA into 100% serum using a Tecan EVO Freedom (Tecan, Männedorf, Switzerland) workstation and were stored at −70 ± 10 °C.

2.2. Serum Specimens

Control cynomolgus monkey and rat serum samples were obtained from Bioreclamation Inc., (Hicksville, NY, USA). Serum samples were stored at −70 ± 10 °C once they were received. Individual serum lots were used in matrix screening, for preparation of standard and QC, and in selectivity experiments. Once the individual serum lots were screened against standard curve prepared in buffer, serum lots that were within normal distribution of the readout (mean ± 2SD) were pooled and used for standard and QC preparation in serum for pre-study and in-study validations.

3. Methods

The general procedure for the 3 methods is depicted in the flow diagram in Fig. 2, with the details of reagents used listed in Table 1. The assay buffer was I-Block with 1 M NaCl for method A; and 1X Dubelco's phosphate buffer saline (DPBS) plus 1% bovine serum albumin (BSA), 1 M NaCl, and 0.5% Tween 20 for method B and C. Microplate wells were coated with the corresponding capture reagent in 1X DPBS for each method as listed in Table 1. Plates were blocked for 1 to 3 h. Sample incubation time for method A and B/C was 2 ± 0.16 h and 30 ± 10 min, respectively. Detection antibody incubation time was 1 ± 0.16 h for all 3 methods. Secondary antibody incubation time in Method A was 30 ± 5 min. The signals were read on a Sector Imager 6000.

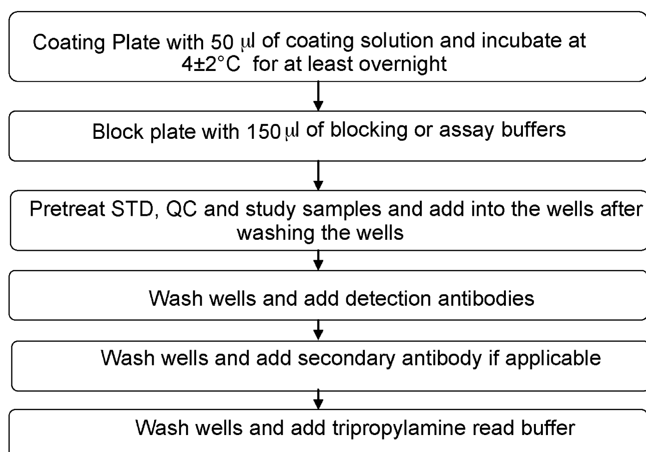

Fig. 2. General flow diagram of methods.

3.1. Automation instrument and equipment

The Tecan EVO Freedom workstation equipped with Evoware software was used to prepare STD, VS, and QC. A worklist file containing dilution information of STD, VS, or QCs was created using Microsoft Excel. A script was developed for the workstation to read the worklist and execute the dilution process. A Meso Scale Discovery sector imager 6000 equipped with Discovery workbench software was purchased from MSD®. Watson LIMS™ (Thermo Scientific, MA, USA) was used for data acquisition.

3.2. Instrument validation

The installation and operation qualification of the MesoScale 6000 platform and interface to the Watson LIMS were performed using standard procedures. The modular automated scripts and processes of preparation and sample dilution in Tecan EVO Freedom were validated [11].

3.3. Method optimization

After method feasibility, 2 optimization runs were performed using 2 independent preparations of STD without VS in the run for methods A and B, and 3 optimization runs were performed using 2 independent preparations of STD without VS in the run for method C.

3.4. Pre-study validation

3.4.1. Accuracy and precision experiments

Robustness and ruggedness of the method were tested by having at least 3 technicians performing a minimum of 6 accuracy and precision runs over 4 days at conditions of short and long incubation times using different equipments (i.e. different pipettes, plate washers, plate readers and incubators). At least 2 independent sets of STD were prepared using a Tecan robotic workstation. For methods A and B, the standard curve consisted of 10 standard points, 2 of which were anchor points, and a blank. For method C, a standard curve consisted of 11 standard points, 2 of which were anchor points, and a blank serum. Each accuracy and precision run included one set of STD and at least 4 replicates of VSs at concentrations of LLOQ (lower limit of quantification), LQC (low level QC), MQC (mid level QC), HQC (high level QC), and ULOQ (Upper limit of quantification). The performance characteristics of accuracy (% bias) and precision (% coefficient of variance [CV]) were calculated by a statistical tool (6). The data were used to set the *a priori* in-study run acceptance criteria for STD and QCs.

3.5. In-study validation

Each method was used to support a TK study. In-study STD and QC were also prepared using a Tecan Evo robotic workstation. Each analytical run included one set of STD and at least 2 replicates of QCs at LQC, MQC, and HQC levels. To accept each analytical run, the following criteria must be met: (1) at least 75% of the back-calculated standard value must be within 15% of the nominal value, and (2) 4 out of 6 QCs must be within 15% of the nominal value (4-6-15 rule). The acceptance criteria were determined from accuracy and precision experiments for each method.

3.6. Regression model, statistical approaches and software

A five-parameter logistic (5PL) regression model was used to fit the concentration-response for the standard curves of all 3 methods. The intra- and inter-assay performance characteristics, including total error (sum of % bias and % CV) were calculated from pre-study accuracy and precision experiments using a validated Ligand Binding Assay's EXCEL Software program. For in-study assay performance, the accuracy and precision result was generated by validated Watson™ LIMS system.

4. Results

4.1. Truncation of assay ranges from method optimization to pre-study validation

During the feasibility and qualification tests of the methods, relatively wide assay ranges (between 2 and 3 logs) were observed (Figs. 3A and 5B). Because the assay accuracy at the high concentration region tends to be higher than 10%, the ranges at the high end were truncated by approximately 50%. The low end of assay range (sensitivity) was similar for method A during optimization and pre-study validation. However, the LLOQ needed to be raised to 1/3 for method B or 1/2 for method C. Table 2 shows the potential assay ranges and sensitivity during method optimization versus that of the method validation. Figs. 3–5 show the assay variability of the methods over the entire ranges for all three methods during method optimization (Figs. 3A, 4A, and 5A) and pre-study validations (Figs. 3B, 4B, and 5B). The sensitivity and assay ranges were method dependent. In general, the plots of the method optimization data indicated less acceptable assay performance at the high concentration region. To improve method accuracy and precision for reliable performance, assay ranges were truncated by least 50% in all three methods.

Table 2
Summary on method optimization and validation assay ranges.

	Assay development		Pre-study validation	
	Potential assay range (ng/mL)	Potential assay sensitivity (ng/mL)	Validated assay range (ng/mL)	Validated assay sensitivity (ng/mL)
Method A	50–6000	50	50–2500	50
Method B	50–20,000	100	150–10,000	150
Method C	100–80,000	100	200–40,000	200

Table 3
(A–C) Percent relative error (average %RE) in each standard and validation samples with different weighting factors.

	Weighting factor	%RE in each standard curve concentration (ng/mL)								%RE in each validation sample concentration (ng/mL)				
		50	100	250	500	1000	1400	2000	2500	50	140	400	1800	2500
Method A	1	−18	−3	−2	1	0	1	−1	1	−24	−3	1	−1	−1
	1/Y	3	−1	−2	0	0	1	−1	0	−1	−3	1	−1	−1
	1/Y2	3	−1	−2	1	1	1	−1	0	−1	−3	1	−1	−1
	1/F	3	−1	−2	0	0	1	−1	0	−2	−3	0	−1	−1
	1/F2	3	−2	−2	0	0	1	−1	0	−1	−3	1	−1	−1

	Weighting factor	%RE in each standard curve concentration (ng/mL)								%RE in each validation sample concentration (ng/mL)				
		150	300	600	1250	2500	5000	7500	10000	150	400	4000	7200	10000
Method B	1	−51	−13	10	6	1	−1	0	1	−38	10	1	−2	3
	1/Y	4	5	6	0	−2	−2	1	2	8	12	−1	−1	4
	1/Y2	2	−1	1	−2	−1	1	3	3	6	12	2	1	5
	1/F	1	3	5	0	−2	−2	1	2	5	10	0	−1	4
	1/F2	3	0	1	−4	−5	−2	6	0	7	11	−1	2	24

	Weighting factor	%RE in each standard curve concentration (ng/mL)											%RE in each validation sample concentration (ng/mL)				
		100	200	400	1000	2500	5000	10000	18000	32000	40000	62000	200	600	10000	30000	40000
Method C	1	147	61	3	−1	1	0	2	0	−2	2	−1	65	6	4	4	1
	1/Y	0	0	−1	−1	1	−1	2	−1	−2	3	−1	7	3	4	5	1
	1/Y2	0	0	−1	0	3	1	1	−2	−4	2	10	7	4	4	2	0
	1/F	−2	1	0	0	2	−1	1	−2	−3	2	1	7	4	3	4	1
	1/F2	−2	1	1	−1	1	−1	1	−2	−1	1	2	10	6	2	3	0

5-parameter logistic regression model was used in each method. For each weighting factor evaluation, %RE was averaged from 6 qualification runs performed prior to pre-study validation were averaged to confirm the appropriateness of fit model.

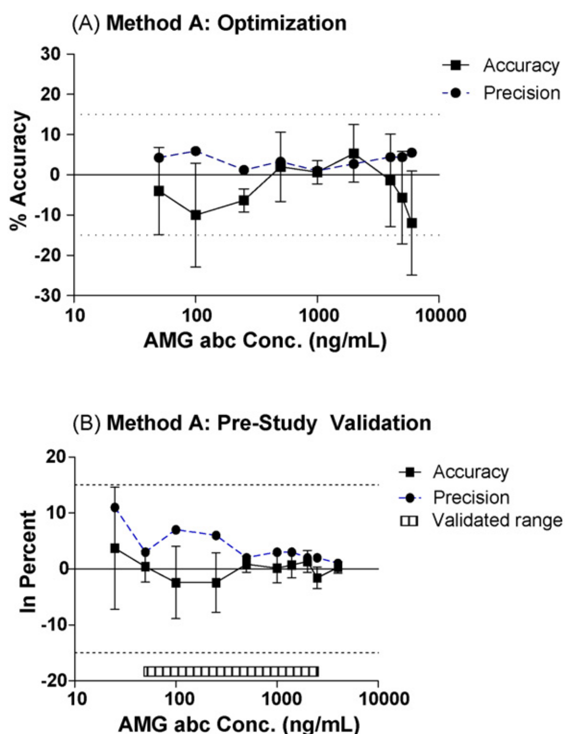

Fig. 3. Standard curve performance of method A observed during method optimization (3A) and pre-study validation (3B). Percent accuracy (% bias) was calculated from the back-calculated concentration from the nominal value of each standard. (A) The mean accuracy and precision at each standard point (mean from 3 runs) with 95% confidence interval. Potential assay ranges were based on a target of 15% accuracy (dotted line). (B) The mean accuracy (filled square with solid line) at each standard point from 7 accuracy and precision runs with 95% confidence interval. Filled circle ( ) with dotted line represents the % CV (precision) from same 7 runs.

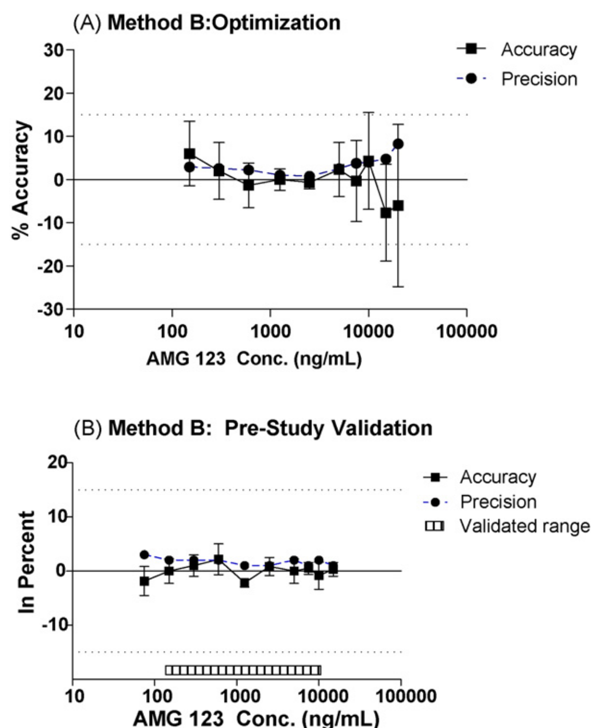

Fig. 4. Standard curve performance of method B observed during method optimization (4A) and pre-study validation (4B). Percent bias at each standard point from its nominal concentration was calculated from the back-calculated concentration of standard during the pre-study validation. Fig. 4A illustrates the mean accuracy and precision at each standard point (mean from 3 runs) with 95% confidence interval. Potential assay ranges were based on a target of 15% accuracy (dotted line). Fig. 4B illustrates the mean accuracy (filled square with solid line) at each standard point from 6 accuracy and precision runs with 95% confidence interval. Filled circle with dotted line represents the % CV (precision) from same 6 runs ( ).

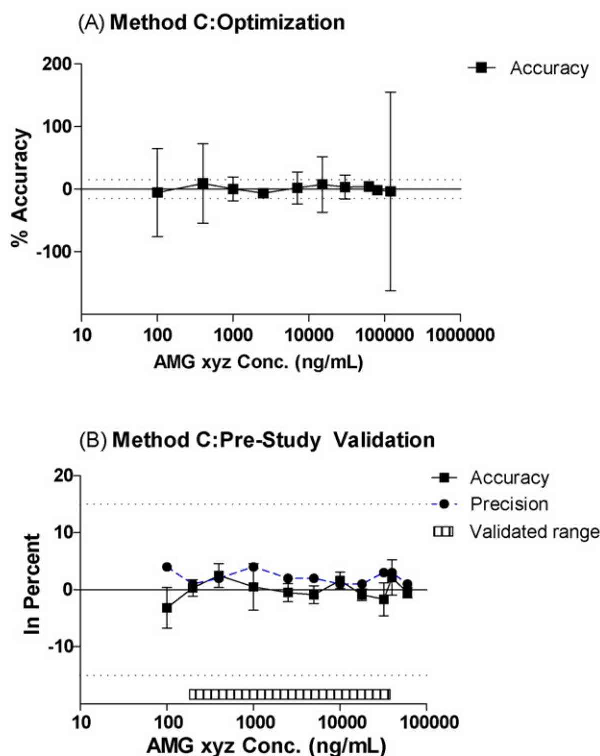

Fig. 5. Standard curve performance of method C observed during method optimization (5A) and pre-study validation (5B). Accuracy at each standard point from its nominal concentration was calculated from the back-calculated concentration of standard during the pre-study validation. Fig. 5A illustrates the mean accuracy precision at each standard point (mean from 2 runs) with 95% confidence interval. Precision profile was not determined for $N=2$. Potential assay ranges were based on a target of 15% accuracy (dotted line). Fig. 5B illustrates the mean accuracy (filled square with solid line) at each standard point from 6 accuracy and precision runs with 95% confidence interval. Filled circle with dotted line represents the % CV (precision) from same 6 runs.

After truncation, the goodness-of-fit model was evaluated using full standard calibrators and validation samples at 5 levels in all three methods. Percent relative error (%RE) from back-fitted or back-calculated concentration was determined using different weighting factors (1, $1/Y$, $1/Y^2$, 1/F or $1/F^2$). In general, %RE in both standard and validation samples of all three methods were highest at the lower end of the standard curve calibration when no weighting was used (Table 3A–C). The result indicated that the weighting factor was necessary to maintain the assay sensitivity. In comparing the different weighting factors of $1/Y$, $1/Y^2$, $1/F$ or $1/F^2$, there was not much difference in %RE, thus $1/Y$ was selected to proceed in pre-study validations.

Truncation of the assay range along with the goodness of fit ($1/Y$) resulted in much tighter accuracy and precision data during pre-study validation. Inter-assay accuracy for the standard was <5% across the standard curves in all three methods (Figs. 3B, 4B, and 5B). Inter-assay precision for the standard was less than 7% for method A, and was 2% and 4% for methods B and C, respectively. Based on the accuracy and precision results, the assay acceptance criterion for the back-calculated standard value was set at 15% for each of the 3 methods.

4.2. Accuracy and precision of validation samples during pre-study validation

For method A, inter-assay accuracy for VS ranged from −5% to −1% and inter-assay precision ranged from 2% to 7%. The total error in the VS was less than 9% (Fig. 6A). For method B, inter-assay accuracy for VS ranged from −3% to 3% and inter-assay precision ranged from 6% to 10%. The total error was less than 14% (Fig. 6B). For method C, inter-assay accuracy for VS ranged from −1% to 3% and inter-assay precision ranged from 5% to 12%. The total error was less than 14% (Fig. 6C). Based on the accuracy and precision results, assay acceptance criterion for QC was set at 15% total error for all 3 methods.

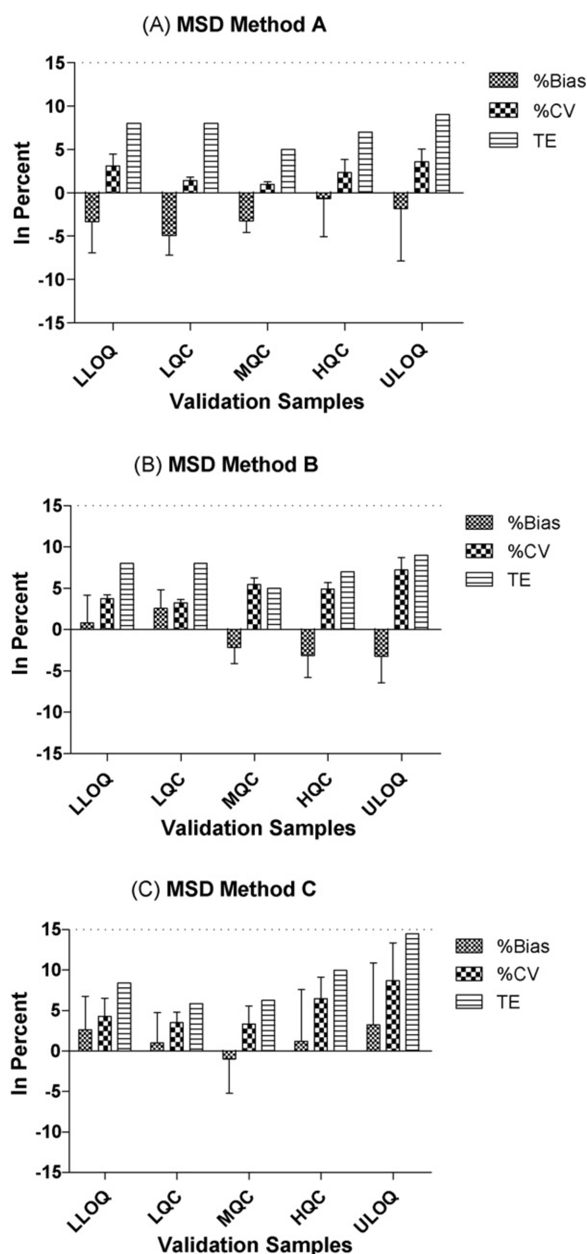

Fig. 6. The assay accuracy and precision in 3 methods were determined by assessing both systemic and random errors using validation samples. Accuracy and precision of the employed method were assessed using 5 levels of validation samples (LLOQ, LQC, MQC, HQC, and ULOQ) in each method. Accuracy of each validation sample from its nominal concentration was calculated from the standard curve during the pre-study validation. Dotted line indicates the 15% acceptance limit and the error bar in % bias (accuracy) and % CV (precision) represented the 95% confidence interval. Total error was the sum of accuracy and precision.

Table 4
In-study inter-assay performance statistics and assay success rates.

	In-study validation performance					
	In-study method performance statistics					In-study
	%Bias of STD	%CV of STD	% Bias of QC	%CV of QC	Total error %	Assay passing rate* (%)
Method A	−2 to 1	1 to 5	−1 to 2	4 to 5	6	95
Method B	−1 to 1	2 to 4	−2 to 1	5 to 11	6 to 12	91
Method C	−2 to 4	2 to 5	−1 to 4	4 to 7	5 to 10	94

*Passing criteria: total number of assays where at least 75% of the standard points must meet 15% accuracy and 4 out of 6 QCs meet 15% total error as determined from accuracy and precision experiments for each method.

4.3. Accuracy and precision performance during in-study validation

For method A, 2 technicians analyzed the study samples from the dosing phase over 11 days and only one technician (1 of 2 technicians from dosing phase) completed analysis for the rest of the samples from the recovery phase in 3 days. A total of 37 runs were performed, and 35 out of 37 successfully met the method acceptance criteria. The average bias for STD and QC ranged from −1 to 1, and precision ranged from 2 to 4 (Table 4). Total error was 6% at all three QC levels.

For method B, 1 technician conducted the dosing phase sample analysis over 13 days. The analysis of recovery samples were performed by the same technician plus an additional technician in 5 days. A total of 51 runs were performed, and 46 out of 51 met the method acceptance criteria. The average bias ranged from −2 to 1, and precision ranged from 5 to 11 (Table 4). Total error ranged from 6% to 12% among 3 QC levels.

For method C, 1 technician conducted the dosing phase sample analysis over 9 days and recovery samples over 2 days. A total of 36 runs were performed, and 34 out of 36 met the method acceptance criteria. The average bias ranged from −2% to 4%, and precision ranged from 2% to 5% (Table 4). Total error ranged from 5% to 10% among 3 QC levels.

The overall in-study performance success rate was >90% for the 3 methods.

5. Discussion

We have presented results from method optimization and pre-study and in-study validations of 3 different methods that apply non-bead ECL technology. The ECL reagents were used with either target protein or anti-idiotypic monoclonal antibodies in sandwich ELISA formats. ECL was chosen because of its sensitivity and potential for having a wider dynamic assay range than conventional ELISAs (6 logs versus 2 logs). These features were observed during method feasibility tests and were also seen in the optimization data for all 3 methods. However, imprecision at the upper end of the assay range resulted in reduced accuracy and precision. The upper assay range was therefore intentionally truncated by at least 50% to increase the confidence in the accuracy and precision performance of each method. Thus, a wide dynamic range could not be achieved to meet the accuracy and precision requirements of regulated PK/TK studies. Truncation of assay ranges improved the accuracy and precision performance at the lower assay range, most notably for methods B and C. The results also confirmed that the most suitable regression model for the fitting process was applied [12]. Accurate and precise determinations are essential, especially at high concentration ranges, because these values may substantially contribute to the TA exposure evaluation. With the truncated assay ranges, TK samples at or near C_{max} (from 300 mg/kg dose group) were diluted 1:10000, 1:1000, and 1:100 for methods A, B, and C, respectively, during in-study validations. Application of robotic workstations will be the next step in minimizing the dilution error for study samples that require greater than 100-fold dilution. Integration of robotic applications to retrieve, execute, and interface the dilutional information from Watson LIMS that is currently being validated for regulated study support.

Based on these results, a few advantages are evident in using planar ECL methods. Performance of standards and QCs in all 3 methods during in-study validation was correlated with those from the pre-study validations. All 3 methods were validated for their intended purposes. The in-study assay success rate for all 3 methods was >90%. The outcome of in-study method performance was the culmination of the rigorous processes of thorough ECL method development and optimization, pre-study validation, and incorporation of automation. Although these pre-study and in-study results may seem impressive compared with those of conventional colorimetric ELISA methods, there were limitations associated with the planar ECL platform. The major limitations include financial and technical challenges such as reagent cost and single source of supplier for those reagents, which could lead to a single point of failure for support of a drug development program. Discontinuation of the supply of reagents and instruments for a bead-based ECL platform has previously affected pharmaceutical customers using other bead-based ECL platforms for drug development [13]. For this reason, conventional ELISA remains the preferred technique of bioanalytical laboratories in supporting regulated studies for drug development. One strategy is to develop methods with planar ECL when the sensitivity of the method is critical for study support and when the methods are needed for short-term support rather than long-term support. The current study highlighted the use of non-bead based ECL assays in regulated studies that are supported for 2 to 3 years. If a longer time frame is required for study support, there should be a back-up plan because of the possibility of reagent supply discontinuation.

In summary, ECL technology may be highly beneficial in quantification of macromolecules in support of PK/TK studies if the availability of reagents from a single source can be proactively addressed with backup suppliers to prevent single point of failure. Alternatively, this technology may be limited to use in short-term regulated studies. Additionally, some planar ECL methods cannot be developed to cover a wide range, as was evident from the results presented herein. The choice of reagents and format design may vary, depending on the study purpose and long-term requirements of method robustness and reproducibility. Strategies should be developed to exploit the use of new technologies supporting PK/TK studies at the early stages and conventional methods for late-stage outsourcing. However, this may necessitate method cross-validation. Careful planning and cross-validation of method transitions and laboratory transitions must be carried out based on PK assessments.

6. Conclusions

Three planar ECL-based methods were developed, optimized, and validated to support PK/TK studies. The desirable wide assay

dynamic range initially sought could not be achieved without sacrificing accuracy at the upper end of the assay range. The methods were therefore optimized with more limited assay ranges, which depended on the analytes, reagents, and the intended use of the methods (regulated versus exploratory studies). Truncation of the assay range at the upper end before the pre-study validation was necessary, since proper method development and optimization are crucial for regulated studies. Our results showed the benefits of all 3 planar ECL methods in terms of accuracy and precision. Combined with the use of automation, the total error of the methods was less than 10% in both pre-study and in-study validations. Further studies will be needed to confirm the advantages of planar ECL-based methods in regulated studies. Based on the current study, however, the technology demonstrated reliably high performance in support of PK/TK studies, albeit with reservations about the risk of single-source supply.

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