Bioanalysis

ISSN: 1757-6180 (Print) 1757-6199 (Online) Journal homepage: www.tandfonline.com/journals/ibio20

Comprehensive Performance Evaluation of Ligand-Binding Assay–LC–MS/MS Method for Co-Dosed Monoclonal Anti-SARS-CoV-2 Antibodies (AZD7442)

Yue Huang, Michael Shane Woolf, Chun-Chi Wang, Sami M Naser, Aaron M Wheeler, William R Mylott Jr, Eric Ma & Anton I Rosenbaum

To cite this article: Yue Huang, Michael Shane Woolf, Chun-Chi Wang, Sami M Naser, Aaron M Wheeler, William R Mylott Jr, Eric Ma & Anton I Rosenbaum (2024) Comprehensive Performance Evaluation of Ligand-Binding Assay–LC–MS/MS Method for Co-Dosed Monoclonal Anti-SARS-CoV-2 Antibodies (AZD7442), Bioanalysis, 16:7, 149-163, DOI: 10.4155/bio-2023-0225

To link to this article: https://doi.org/10.4155/bio-2023-0225

© 2024 Expert Publishing Science Ltd trading as Taylor & Francis

View supplementary material

Published online: 22 Feb 2024.

Submit your article to this journal

Article views: 2577

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at https://www.tandfonline.com/action/journalInformation?journalCode=ibio20

Research Article

For reprint orders, please contact: reprints@future-science.com

Bioanalysis

Comprehensive performance evaluation of ligand-binding assay–LC–MS/MS method for co-dosed monoclonal anti-SARS-CoV-2 antibodies (AZD7442)

Yue Huang[1], Michael Shane Woolf[2], Chun-Chi Wang[3], Sami M Naser[2], Aaron M Wheeler[2], William R Mylott Jr[2], Eric Ma[2] & Anton I Rosenbaum*,[1]

[1]Integrated Bioanalysis, Clinical Pharmacology & Safety Sciences, R&D, AstraZeneca, South San Francisco, CA 94080, USA

[2]Chromatographic Services – Research & Development, Biologics by LC–MS/MS, PPD Laboratory Services (a part of Thermo Fisher Scientific), Richmond, VA 23229, USA

[3]Integrated Bioanalysis, Clinical Pharmacology & Safety Sciences, R&D, AstraZeneca, Gaithersburg, MD 20878, USA

*Author for correspondence: anton.rosenbaum@astrazeneca.com

Aims: AZD7442 is a combination SARS-CoV-2 therapy comprising two co-dosed monoclonal antibodies. **Materials & methods:** The authors validated a hybrid ligand-binding assay–LC–MS/MS method for pharmacokinetic assessment of AZD7442 in human serum with nominal concentration range of each analyte of 0.300–30.0 μg/ml. **Results:** Validation results met current regulatory acceptance criteria. The validated method supported three clinical trials that spanned more than 17 months and ≥720 analytical runs (~30,000 samples and ~3000 incurred sample reanalyses per analyte). The data generated supported multiple health authority interactions, across the globe. AZD7442 (EVUSHELD) was approved in 12 countries for pre-exposure prophylaxis of COVID-19. **Conclusion:** The results reported here demonstrate the robust, high-throughput capability of the hybrid ligand-binding assay–LC–MS/MS approach being employed to support-next generation versions of EVUSHELD, AZD3152.

Plain language summary: The measurement of antibodies in human body fluids (e.g., blood, serum) has historically been tied to laboratory tests that may face operational limitations, including susceptibility to interference from other blood components and a reliance on unique reagents that can take months to produce. As such, there is a pursuit of alternative analytical methods to more accurately detect and measure antibody drugs from complex matrices. In the method, the authors describe different techniques that once combined were used to capture, separate, filter, fragment and then detect and measure the co-dosed antibody drugs. This method has been validated in accordance with current health authority guidelines and has been used to support three clinical trials that spanned more than 17 months; that is, the validated method was used to analyze nearly 30,000 serum samples from more than 2000 patients. Collectively, the results reported here demonstrate the robustness and high-throughput capability of this analytical approach.

First draft submitted: 27 October 2023; Accepted for publication: 1 February 2024; Published online: 22 February 2024

Keywords: hybrid IA–LC–MS/MS • hybrid LBA–LC–MS/MS • incurred sample reanalysis • monoclonal antibody • pharmacokinetics • SARS-CoV-2 • tixagevimab and cilgavimab • validation

Since 1986, the US FDA has approved more than 100 antibody therapeutics; these products have revolutionized the treatment of numerous diseases [1–4]. Antibody products constitute a considerable portion of the top ten list of best-selling drugs, with global sales exceeding $100 billion annually [5]. To elaborate, ligand-binding assays (LBAs) were historically the pinnacle of bioanalysis for most proteins and protein-based serological assays. In fact, LBAs served as the gold standard platform for monoclonal antibody (mAb) bioanalysis well into the early 2000s [6]. As noted by Zhang *et al.*, the 2007 European Medicines Agency Guidelines on the Clinical Investigation of the Pharmacokinetics of Therapeutic Proteins focused entirely on LBAs and made no mention of LC or MS methods [7]. Despite the general ease of LBA implementation, these serological assays occasionally face operational limitations, which include a susceptibility to matrix effects of endogenous components, cross-reactivity and a reliance on

critical reagents that may be limited or unavailable. For example, LBAs typically require anti-idiotype antibodies for quantification of humanized antibodies from human matrix. A pair of highly selective and specific anti-idiotype antibodies can take months to produce. Driven by a need to address these potential challenges, some investigators have been employing hybrid methods that combine the enrichment capability of affinity-based LBAs with the outstanding selectivity of LC–MS/MS [6,8–10]. Hybrid LBA–LC–MS/MS methods (also called immunoaffinity [IA]–LC–MS) are particularly useful in cases with specificity and critical reagent availability challenges.

Hybrid LBA–LC–MS/MS approaches began appearing in peer-reviewed literature in the late 2000s and early 2010s [6,11,12]. For convenience, the authors have provided additional references to key research articles [13], white papers [14–17], editorials [8,18] and journal special issues [19] that offer further insights into the topic of hybrid IA–LC–MS. However, much of the prevailing literature related to the hybridization of LBAs and LC–MS/MS for large molecule bioanalysis do not center on the absolute quantification of therapeutic mAbs or the application of LBA–LC–MS/MS to large-scale clinical trials. Most of these search results describe sizable antibody studies and report the use of LBAs or LC–MS for the bioanalysis of large and small molecules, respectively. Publications detailing and reviewing hybrid LBA–LC–MS/MS as a bioanalytical platform for quantitation of biotherapeutics and biomarkers are also numerous [20–22]. However, few publications focus on the application of hybrid LBA–LC–MS/MS for the bioanalysis of mAbs [23–25]; fewer still describe long-running, large-scale clinical trials that employ LBA–LC–MS/MS for the bioanalysis of mAbs, such as the POLARIX trial including 879 participants (NCT03274492) [26] (see Supplementary Material for further details on the literature search). The findings presented here follow previous work describing the development of a hybrid LBA–LC–MS/MS method for the absolute quantification of the co-dosed mAbs comprising AZD7442 [9]. In the previous article, the authors reported studies to evaluate assay capture capacity, the potential impact of variable clearance rates of co-dosed mAbs on analyte quantification (i.e., disproportionate analyte concentrations), critical reagent characterization (comparing RBD and anti-triple mutant as a capture reagents) and proactive evaluation of convalescent and vaccinated patient sera to determine if endogenous anti-RBD antibody could compete with AZD1061 and AZD8895 for binding with capture reagent(s) (i.e., assay performance was not adversely impacted by active COVID-19 infection or vaccination-induced anti-RBD IgG). Here the authors report the full validation and post-validation, clinical sample analysis robustness assessment of the hybrid LBA–LC–MS/MS method for quantification of AZD7442 in human serum.

Materials & methods

A detailed accounting of chemicals, reagents, materials, and sample handling methods was reported previously [9]. Briefly, aliquots of human serum were diluted with loading buffer and combined with magnetic beads that were functionalized with RBD protein: RBD protein (receptor-binding domain) was provided by AstraZeneca, while Magne® streptavidin beads were purchased from Promega Corporation, WI, USA. Functionalized beads were used for the immunocapture of mAbs (AZD1061 and AZD8895). After the capture step, the beads were washed, denatured and chemically treated [9]. To generate surrogate (characteristic) peptides for LC–MS/MS quantitative analysis, samples were subjected to on-bead, proteolytic digestion (trypsin). Signature peptide sequences for AZD1061 and AZD8895 were DVWMSWVR and ASGFTFMSSAVQWVR, respectively. After chemically halting the enzymatic digestion (2 N HCl), stable isotope-labeled internal standards were added (DVWMSWV[$^{13}C_6$, $^{15}N_4$-R] or ASGFTFMSSAVQWV[$^{13}C_6$, $^{15}N_4$-R]; Elim Biopharmaceuticals, CA, USA) and eluates were cleaned via filtration. Spectrophotometric absorbance measurements at 280 nm (Beer's Law) were used to determine the concentrations of AZD1061 and AZD8895 reference material stock solutions (AstraZeneca, MD, USA): 30.7 mg/ml tixagevimab (total protein extinction coefficient 1.49 [mg/ml] $^{-1}$cm^{-1}) and 30.2 mg/ml cilgavimab (total protein extinction coefficient 1.56 [mg/ml] $^{-1}$cm^{-1}). Prior to extraction and analysis, calibrators and quality controls were spiked with AZD1061 and AZD8895 reference materials.

Calibrators

Calibrators were prepared in human serum with AZD8895 and AZD1061 concentrations of 0.300, 0.500, 0.800, 2.00, 5.00, 12.0, 24.0 and 30.0 µg/ml. During validation, calibrators were prepared in polypropylene tubes, vortexed and allowed to equilibrate on ice for 10–15 min. Calibrators were prepared fresh for each run.

Quality controls

Quality control (QC) pools were prepared in human serum with AZD8895 and AZD1061 concentrations of 0.300, 0.600, 14.0 and 22.5 µg/ml. Two different over-the-curve QCs were also prepared in human serum: 60.0 µg/ml

Taylor & Francis
Taylor & Francis Group

and 900 µg/ml. Prior to freezing of QC pool daily use portions, all tubes were vortexed and allowed to equilibrate over ice for 10–15 min.

Instrumentation & data collection

Chromatographic separation was achieved using 100:0.1 water/formic acid, v/v as mobile phase A and 100:0.1 acetonitrile/formic acid, v/v as mobile phase B using a reversed-phase analytical column maintained at 45°C (ACQUITY UPLC HSS T3 C18, 2.1 mm × 50 mm, 1.8 µm, product no. 186003538, Waters Corp.). Flow rate varied from 0.35 to 0.45 ml/min. Total run time was 5 min. Positive electrospray ionization (ESI) provided the interface between the analytical column and a Sciex API 6500+ triple quadrupole mass spectrometer (SCIEX, MA, USA). Data from LC–ESI–MS/MS systems were acquired and processed in Analyst (v1.6.2, SCIEX), MultiQuant (v3.0.3, SCIEX) using the MQ4 processing algorithm and Assist laboratory information management system (LIMS) v7 software (PPD, NC, USA).

All graphical displays featured in this manuscript were prepared in Prism v8.3.1 for Windows (GraphPad Software, CA, USA) or in R version 4.2.0 (2022-04-22) using the ggplot2, dplyr, ggdist, forcats and cowplot packages (www.r-project.org) [27,28].

Method validation studies

This assay was successfully validated in human serum in accordance with current regulatory guidance and industry recommendations for bioanalytical method validation [17,29,30]. Validation studies assessed assay linearity, accuracy and precision, specificity/selectivity, cross-analyte interference, analyte stability, reinjection reproducibility, recovery, matrix effect (e.g., hemolysis and lipemia) and carryover. Summary statistics were calculated in Assist LIMS v7. Percent difference from theoretical and percent coefficient of variation were calculated as ($\%DT = [(\mu/A) - 1] \times 100$) and ($\%CV = (\sigma/\mu) \times 100$), where μ is the mean calculated concentration, A is the theoretical concentration and σ is the calculated standard deviation.

Clinical trials

The fully validated method was used to support multiple clinical trials, including the PROVENT (NCT04625725), STORM CHASER (NCT04625972) and TACKLE (NCT04723394) clinical trials [31–33].

Assessment of method robustness

Postdeployment method evaluation encompassed interference studies in convalescent, vaccinated and RBD-spiked sera [9]. Additionally, incurred sample reanalysis (ISR) and assay lifecycle QC performance tracking played vital roles in the assessment of assay robustness. For ISR samples, relative percent difference (RPD) was calculated as

$$RPD = \frac{difference}{mean} \times 100 = \frac{A - B}{(A + B)/2} \times 100$$

where A is the initial reportable concentration and B is the concentration measured during the ISR [34].

Results & discussion

Validation results

The following sections report and discuss validation of the method for absolute quantitation of the co-dosed mAbs comprising AZD7442. These validation studies met acceptance criteria in multiple jurisdictions. The validation data are summarized in Table 1. Following validation, this method was used to support multiple clinical trials. In the 'Assessment of Method Robustness' section, QC and ISR performance trends from three clinical trials were analyzed. QC and ISR data further demonstrated the method robustness over time. ISR trends are evaluated from three perspectives: by study, through time and across the observed concentration ranges. As part of this postvalidation evaluation, the authors highlight options for richer, more granular analysis and visualization of ISR trends.

Linearity, accuracy & precision

Two eight-point calibration curves were included in each validation run (Supplementary Figure 1). The relationship between peak area response ratio (analyte response:internal standard response) and theoretical concentration was

Table 1. Summary of validation study results.

Validation study		Summary			
Run length		96 injections			
Sample volume		20.0 µl			
Standard curve conc.		0.300–30.0 µg/ml			
QC conc.		0.300, 0.600, 14.0 and 22.5 µg/ml			
	Conc. (µg/ml)	AZD1061		AZD8895	
		Coefficient of variation (%)	Difference from theoretical (%)	Coefficient of variation (%)	Difference from theoretical (%)
Calibrator inter-assay statistics	0.3	5.00%	0.36%	10.20%	2.13%
	0.5	7.65%	-0.83%	5.30%	-1.49%
	0.8	5.04%	-0.75%	6.95%	-2.79%
	2	6.15%	2.05%	5.22%	-3.88%
	5	6.43%	1.16%	5.02%	0.19%
	12	5.77%	-1.92%	5.00%	-1.54%
	24	6.63%	0.32%	5.22%	2.06%
	30	8.33%	-0.64%	4.66%	4.39%
QC intra-run statistics	0.3	2.39 to 19.0%	-10.9 to -1.88%	3.58 to 10.5%	-20.8 to 0.0229%
	0.6	3.96 to 6.56%	-6.40 to -2.71%	2.81 to 5.06%	-11.1 to -5.64%
	14.0	2.89 to 6.76%	3.67 to 5.22%	1.82 to 6.89%	-3.42 to 3.01%
	22.5	4.53 to 9.03%	-0.238 to 13.6%	2.06 to 7.66%	-3.35 to 8.17%
QC inter-run statistics	0.3	11.10%	-7.61%	11.80%	-9.57%
	0.6	5.06%	-4.48%	4.68%	-8.77%
	14.0	5.36%	4.58%	5.48%	0.01%
	22.5	8.64%	5.25%	7.04%	3.30%
Overall analyte recovery		123%		100%	
Dilutional linearity		14.0 µg/ml diluted twofold 900 µg/ml diluted 40-fold			
Freeze–thaw stability		Five cycles frozen at -25°C or -80°C and thawed on ice			
Thawed matrix stability		24 h on ice			
Extract stability		102.52 h at 2 to 8°C			
Frozen matrix stability		6 days at -25°C and -80°C			
Solution stress stability in solvent		30.2 mg/ml: 6.02 h on ice		30.7 mg/ml: 6.02 h on ice	
Reinjection reproducibility		Reinjection reproducibility was demonstrated			
Hemolysis		No effect on the quantification			
Lipemia		No effect on the quantification			
Selectivity		No significant interfering peaks in blank human serum			
Matrix factor		Lot-to-lot response consistency was demonstrated			

Conc.: Concentration; QC: Quality control.

linear (1/concentration^2 weighted, least-squares regression) over the nominal concentration range of each analyte (0.300–30.0 µg/ml). Best fit regression models were used to determine empirical concentrations. Across six runs, mean squared coefficients of determination for AZD1061 and AZD8895 were R^2 = 0.9967 and 0.9966, respectively. The lower limit of quantification (LLOQ) was defined as the lowest, quantifiable, nonzero concentration with acceptable accuracy and precision: LLOQ = 0.300 µg/ml.

AZD8895 and AZD1061 QCs were prepared at four concentration levels and analyzed (n = 6): LLOQ and low, mid and high QCs were 0.300, 0.600, 14.0 and 22.5 µg/ml, respectively. These QCs were evaluated across three different runs on different days. Validation intra-run and inter-run data (n = 3 runs with 6 replicates for each QC level) met acceptance criteria at all QC levels (Table 1). That is, at the low-, mid- and high-QCs levels, accuracy and precision were within ±20% mean percent difference from theoretical (%DT) and mean percent coefficient of variation (%CV), respectively; at the LLOQ QC level, accuracy and precision were within ±25% DT and CV, respectively (Figure 1). Analyte carryover was evaluated on a run-by-run basis. Carryover had no significant impact on assay performance.

Taylor & Francis
Taylor & Francis Group

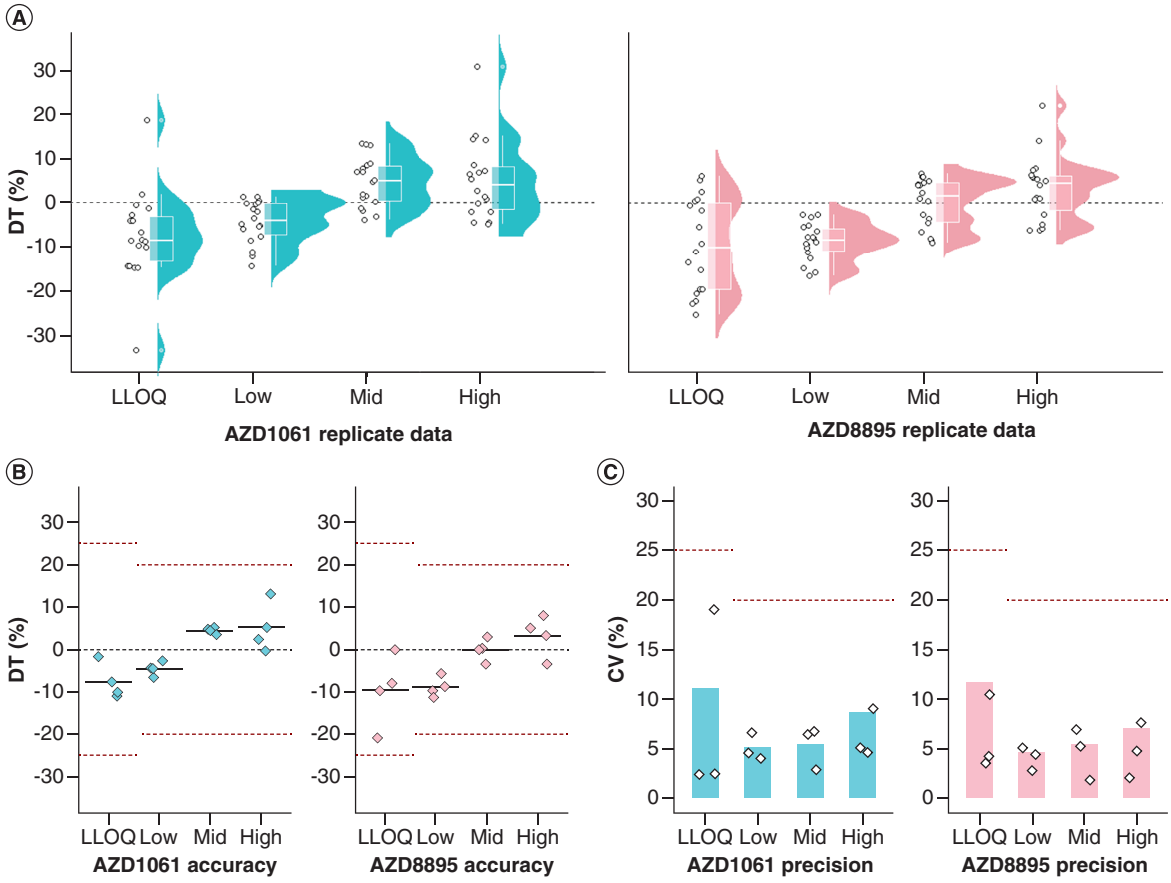

Figure 1. Accuracy and precision for AZD1061 and AZD8895. QCs were prepared at LLOQ, low-, mid- and high-QC levels; n = 6 replicates for each QC level per run (n = 3 validation runs). **(A)** Individual accuracy values for all QC replicates are depicted in raincloud plots consisting of jittered univariate dot plots, Tukey box-and-whisker plots and density (half-eye) plots. **(B)** Assay accuracy. Intra- and inter-run accuracy values: colored diamonds and solid black line, respectively. **(C)** Assay precision. Intra- and inter-run precision values: white diamonds and solid bars, respectively. LLOQ: Lower limit of quantification; QC: Quality control; DT: Difference from theoretical.

Dilutional linearity samples consisted of two sample types intended to mimic real-world clinical samples with either insufficient volume to form a complete aliquot (<20 µl) or initial concentrations well above the upper limit of quantitation (ULOQ). Over-the-curve QCs (900 µg/ml) were diluted 40-fold and processed in replicate (n = 6; Table 1). Simulated insufficient-volume samples (14.0 µg/ml) were diluted twofold and processed in replicate (n = 6). In both instances, diluting samples did not adversely impact assay performance with an accuracy range between 3.31% and 14.2% and a precision range between 3.75% and 5.51% (Figure 2C). .

Analyte recovery in human serum

During one validation run, the authors evaluated the analyte recovery efficiency associated with immunocapture. This apparent recovery study compared the analyte responses of precapture-fortified samples with those of postcapture-fortified samples (samples representing 100% recovery that were spiked just prior to the sample digestion). Recovery samples were analyzed in triplicate (n = 3) at the low-, mid- and high-QC levels. Recovery data were consistent across all QC levels with overall percent recoveries for AZD1061 and AZD8895 of 100% and 123%, respectively (Table 1). Alone, these recovery studies are not a direct indication of assay accuracy but rather a reflection of the appropriateness of the capture reagents and beads. In the authors' experience, it is not unusual to observe recoveries >100% for recovery studies that employ internal standards that are added postdigestion (i.e., internal standard [IS] does not experience immunocapture or digestion) and samples that are fortified postimmunocapture. Importantly, the intra- and inter-assay precision and accuracy results clearly demonstrated

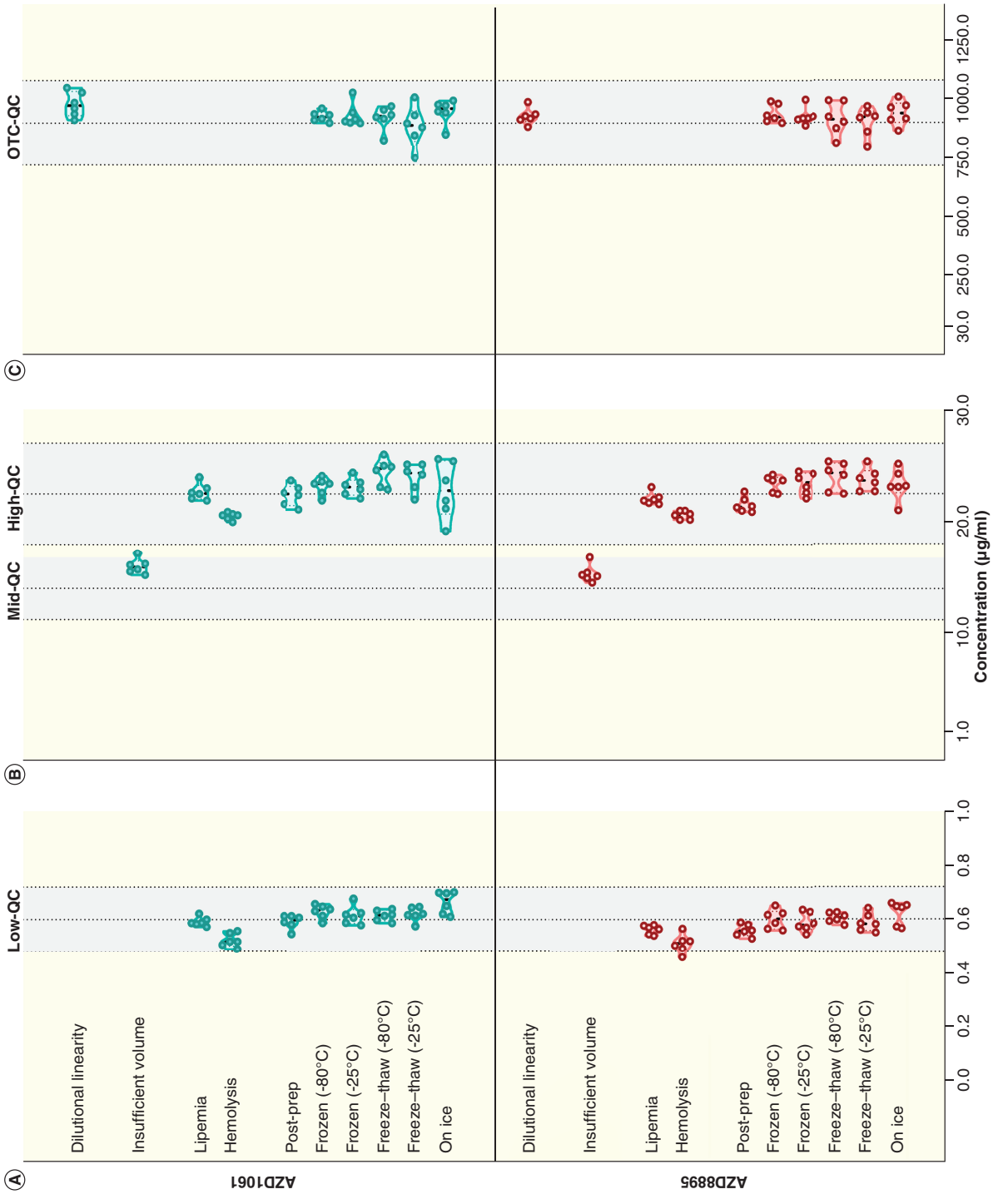

Figure 2. Stability and dilutional study results. Stability data at low (0.600 µg/ml), mid (22.5 µg/ml) and over-the-curve (900 µg/ml) concentrations. Shaded grey bands denote the acceptance ranges for these studies (±20% from theoretical). **(A–C)** Stability stress conditions for samples that were thawed and stored on ice for 24 h, exposed to five freeze–thaw cycles from -25°C or -80°C, stored for 6 days at either -25°C or -80°C or refrigerated at 2–8°C for prolonged periods (postprep). **(B)** Data points for insufficient volume at the mid-QC level, 14 µg/ml diluted twofold. **(C)** Data points for dilutional linearity at the over-the-curve quality control level, 900 ng/ml diluted 40-fold. Post-prep: Post-preparation; QC: Quality Control.

Taylor & Francis
Taylor & Francis Group

that both immunocapture and digestion efficiency are consistent and reproducible across the validated calibration range.

Specificity & selectivity

Unfortified specificity samples consisted of double (matrix without IS) and single blanks (matrix with IS) from six lots of human serum (n = 1 each per lot) that were extracted and analyzed in singlicate. These unfortified specificity samples met acceptance criteria with no appreciable, interfering chromatographic peaks at the retention times of the ISs or the targeted, characteristic peptides: IS response in double blanks ≤5% of the mean single blank IS response and response at the expected retention times of the characteristic peptides were ≤20% of the mean peak area response of the LLOQ.

Fortified specificity samples were prepared by spiking human serum (six lots) with the appropriate drug (AZD1061 or AZD8895) at the LLOQ (0.300 µg/ml). These samples were processed in triplicate (n = 3) and in parallel with the unfortified specificity samples. Acceptance criteria for fortified specificity samples required that for five of the six serum lots, the read-back concentration for two-thirds of the replicates be within ±25% of theoretical concentration at the LLOQ: between 0.225 ng/ml and 0.375 ng/ml (Supplementary Figure 2). Together, these findings indicate that no interfering impurities or co-eluting components were present in different matrix lots and that the ability to consistently quantify analytes at the LLOQ was not adversely impacted by lot-to-lot matrix variation.

Hemolyzed QCs were prepared by fortifying human serum QCs with whole hemolyzed human blood such that the final matrix was representative of 5% hemolysis. Lipemic QCs were prepared in human serum with triglyceride concentration >300 mg/ml. Single and double blanks were processed with hemolyzed and lipemic QC samples at low and high level, n = 6 replicates each. When total sample hemolysis was ≤5% or when sample triglyceride concentration exceeded 300 mg/dl, no impact on analyte quantification was observed (within ±20 %DT and %CV; Figure 2A, B & Table 1). Matrix effect was consistent across all serum lots. Low- and high-level matrix factor QCs in six lots of human serum (four normal, two hemolytic and two lipemic; n = 1 each) met acceptance criteria and exhibited peak area responses across all lots that were <20% of the LLOQ (Table 1).

When blank human serum was fortified with a single analyte at the ULOQ (1000 µg/ml) or IS (level of use), no substantial cross-analyte interference peaks were observed. Cross-analyte performance was considered satisfactory if interference peak contribution to the analyte responses was <20% of the mean peak response at the LLOQ. Similarly, interference peak contribution to the IS responses was acceptable, i.e., <5% of the mean peak response for the high-QC IS for that run.

Stability (short-term, reinjection, long-term & bridging experiment)

General matrix-related analyte stability was assessed in replicate (n = 6) at low, high and over-the-curve QC (900 µg/ml) levels. Stability stress conditions included samples that were stored frozen, exposed to multiple freeze–thaw cycles between thawed and frozen at either -25 or -80°C, thawed and stored on ice or extracted and refrigerated (postpreparative). All stability study data met acceptance criteria across all experimental stress conditions: %CV range between 2.39 and 10.9; %DT range between -6.72 and 10.5. These findings are summarized in Figure 2 & Table 1. Briefly, target analytes were stable for at least 24 h when thawed, for 6 days when stored frozen at -25 or -80°C and for five freeze–thaw cycles. When compared against a freshly prepared calibration curve, the postpreparative AZD1061 samples (refrigerated at 2–8°C for 102.52 h) did not meet acceptance criteria: %DT >20%. In the authors' experience, this is typically due to batch-to-batch or run-to-run variation of analyte/IS ratio. To address this issue the authors compared aged sample data against a calibration curve injected on the day the postpreparative samples were extracted; when evaluated this way, postpreparative samples met general accuracy and precision acceptance criteria with %DT and %CV within ±20% (Figure 2A & B) [35,36]. The authors also evaluated the stability of analytes, and the corresponding ISs, in stock solutions, such as AZD1061 IS at 100 µg/ml in 50:50 acetonitrile/water, v/v. When stored on ice, analytes and ISs demonstrated stability in stock solutions for 6.02 and 5.63 h, respectively. To demonstrate that an analytical run can be successfully reinjected and reanalyzed, reinjection reproducibility samples (n = 2 replicates for each of the eight calibrators) were held in the autosampler at 2–8°C and reinjected after the preceding injection sequence. These reinjection reproducibility samples met all previously stated run accuracy and precision acceptance criteria.

Table 2. Incurred sample reanalysis data summary.

Analyte	Run count	Run pass	Sample count	ISR count	ISR (%)	Cumulative ISR pass rate (%)
AZD1061	721	676	27908	2997	10.74	98.17
AZD8895	726	678	28292	2938	10.39	98.27

ISR: Incurred sample reanalysis.

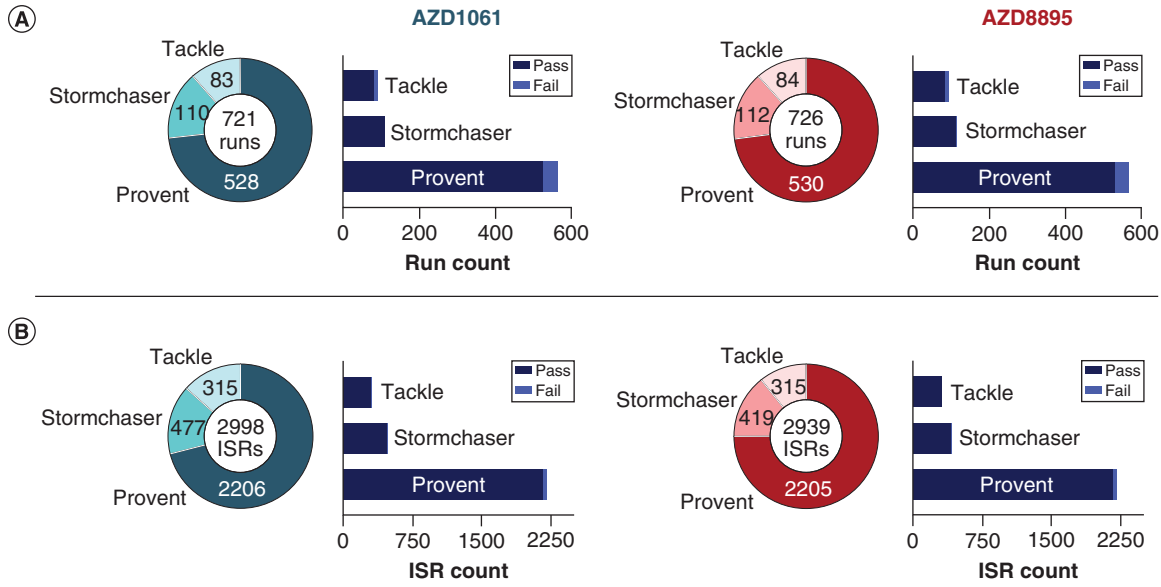

Figure 3. Details of analytical runs and incurred sample reanalysis trends across the three clinical trials. (A) Distribution of analytical runs across all three clinical trials. Stacked bar plots showing run performance (pass/fail) by clinical trial. **(B)** Distribution of ISR samples across all three clinical trials. Stacked bar plots showing ISR performance (pass/fail) by clinical trial.
ISR: Incurred sample reanalysis.

Assessment of method robustness

The authors used the fully validated bioanalytical method to process and analyze samples in multiple clinical trials. This section presents postvalidation reproducibility and robustness data from nearly 30,000 samples in three clinical trials: PROVENT (5197 participants) [37], STORM CHASER (1121 participants) [38] and TACKLE (1014 participants; Table 2) [39]. These trials spanned more than 17 months (from April 2021 through October 2022), encompassed at least 720 analytical runs and included nearly 3000 ISRs from more than 2000 patients (across both analytes, there were 2107 unique patient IDs associated with these ISRs; Figure 3A & C).

QC performance trends

Performance over the assay lifecycle is dependent upon the consistency of system operating conditions, biological components and chemical reagents. Unexpected changes to these variables can cause assay drift leading to quantification bias [40]. Accordingly, after deployment of this fully validated method, run acceptance QCs were used as primary indices for run, system and assay performance (Table 2). At the study level, the methods for AZD1061 and AZD8895 were used to process and analyze 721 and 726 analytical runs, respectively; the PROVENT trial comprised about 73% of these runs, while TACKLE and STORM CHASER accounted for approximately 11 and 15% of the runs, respectively (Figure 3A). In Figure 4, low-, mid- and high-QC performance trends are plotted against run index [40,41]. Acceptance limits for these run QCs were ±20% DT. Low-, mid- and high-QC acceptance pass rates for AZD1061 were 95.86%, 95.71% and 92.60%, respectively. Low-, mid- and high-QC acceptance rates for AZD8895 were 97.93, 97.63 and 94.67%, respectively. Throughout the method lifecycle, these QC performance trends were consistent and showed no appreciable degradation or shifts in the assay behavior or performance. Notably, data presented here included work from 24 different analysts (includes aliquoting, ex-

Taylor & Francis
Taylor & Francis Group

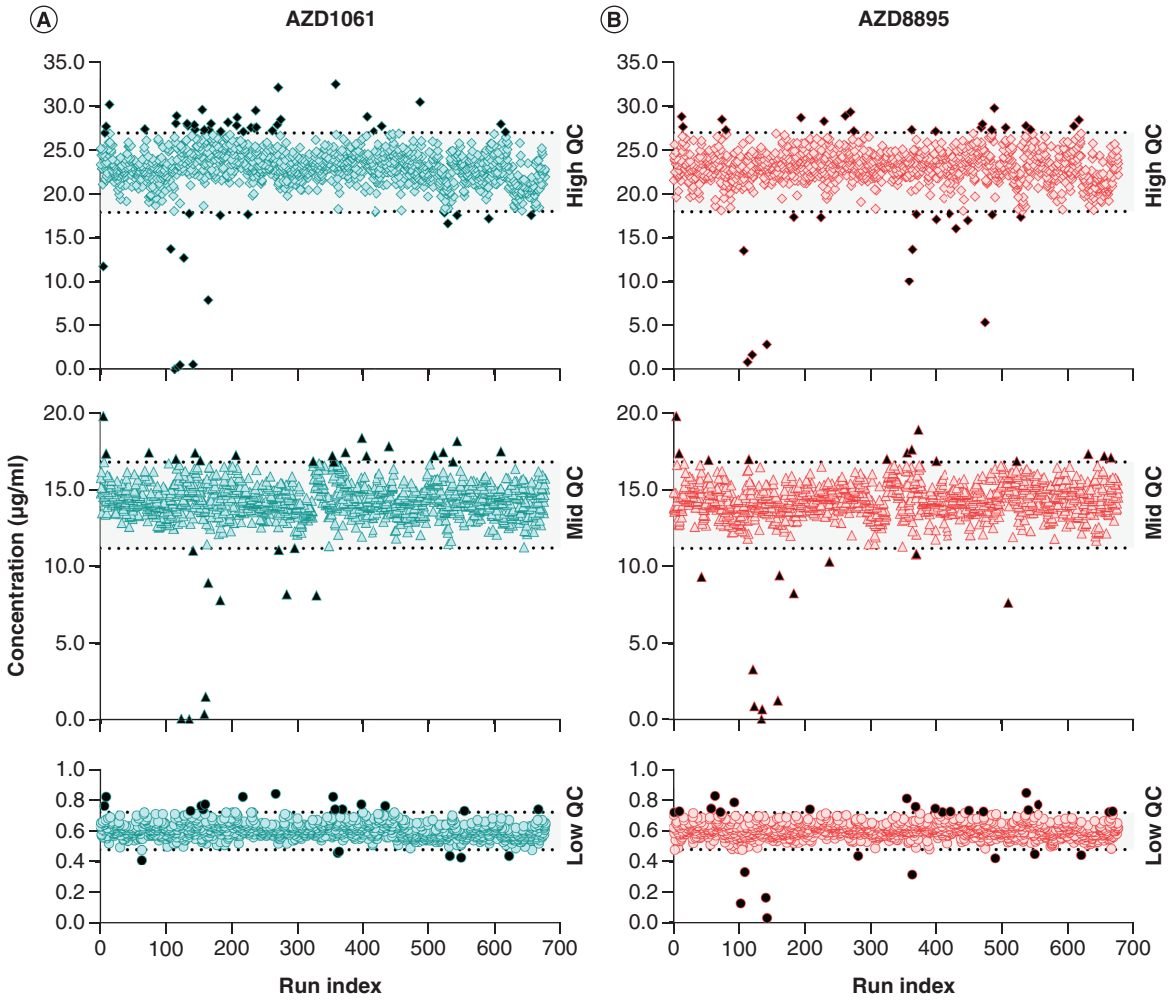

Figure 4. Quality control performance data trends. QC performance datasets for **(A)** AZD1061 and **(B)** AZD8895 include low, mid and high QCs for 676 analytical runs: 0.600, 14.00 and 22.50 µg/ml, respectively. For each analytical run, both replicates at each QC level are plotted. Horizontal dashed lines represent acceptance limits for these QCs (±20% difference from theoretical). Symbols with solid, black fill denote data points that fall outside acceptance limits.
QC: Quality control.

traction and instrument analysts) and from seven different instruments; despite these potential challenges to assay reproducibility, QC performance trends indicate no appreciable changes to assay behavior throughout the method lifecycle.

ISR analysis

In tandem with QC performance trending, ISR is a widely accepted tool for ongoing, postvalidation assessment of bioanalytical method reproducibility and assay robustness. Simply stated, ISR involves the reanalysis of study samples; this reanalysis may occur at irregular intervals and the subset of ISR samples usually constitutes approximately 10% of the original sample set; ISR is required by regulatory guidance [29,34,42]. By serving as an additional in-study validation/process control parameter, ISR provides confidence in long-term method performance and in the data generated throughout the study [43]. For a hybrid LBA–LC–MS/MS method, acceptance criteria required

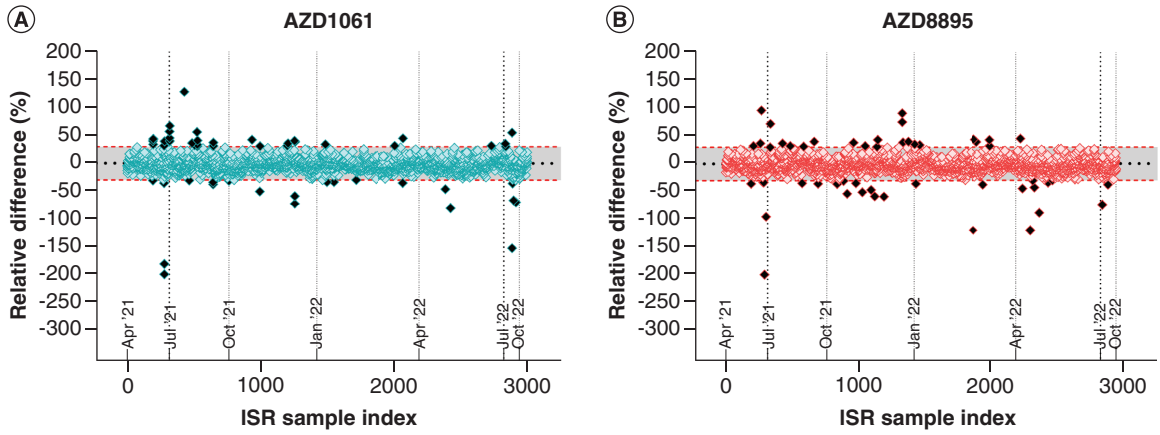

Figure 5. Sequence-dependent (time-dependent) incurred sample reanalysis trend analysis. Relative percent difference as a function of ISR sample index. As of October 2022, ISR pass rates for **(A)** AZD1061 and **(B)** AZD8895 were 98.17 and 98.27%, respectively. Vertical dashed lines appear at 3-month intervals. Black diamonds indicate ISR samples that fell outside acceptance limits.
ISR: Incurred sample reanalysis.

that 2/3 of the ISR samples quantify within ±30 RPD for each analyte (Figures 3 & 5) [34]. At the study level, a total of 2997 AZD1061 ISR samples were analyzed with a cumulative pass rate of 98.17%, while 2938 AZD8895 ISR samples were analyzed with a pass rate of 98.27% (Figure 3). Further, 10.74% of AZD1061 samples were re-analyzed with a mean difference in days between the original analysis and the ISR of 48.16 days (median = 39 days; range = 2–373 days). Likewise, 10.39% of AZD8895 samples were reanalyzed with a mean difference in days between the original analysis and the ISR of 46.33 days (median = 37 days; range = 1–218 days). As time between original analysis and ISR increased, no apparent impact on ISR pass rate was observed (Supplementary Figure 3), further indicating assay robustness over time.

The ICH Harmonized Guideline M10 states that in cases where ISR meets overall acceptance criteria, it may still be advisable to conduct additional evaluations to assess systemic differences in ISR results and to identify underlying analytical issues. Here, sequence- and concentration-dependent ISR evaluations are presented [34]. Across the three clinical trials discussed here (PROVENT, STORM CHASER and TACKLE) AZD1061 ISR pass rates were 98.32, 98.53, and 96.51%, respectively. Similarly, AZD8895 ISR pass rates were 98.37, 97.61, and 98.41%, respectively (Figure 3). Time-dependent (sequence-dependent) ISR trends are presented as plots of RPD as a function of ISR sample index (Figure 5). These sequence-dependent ISR trends were generally unremarkable. Analyte concentration levels in these ISR samples ranged from 0.79 to 51.46 µg/ml, with most ISR sample concentrations below 25 µg/ml (Figure 6A & B). In keeping with the current ICH M10 guidance, these ISRs were selected to provide adequate coverage of the dosed study population concentration profile and are representative of the whole study [34]. Mean and median AZD1061 concentrations were 9.93 and 9.44 µg/ml, respectively. Mean and median AZD8895 concentrations were 10.30 and 9.84 µg/ml, respectively. Histograms of these ISR samples showed right-skewed frequency distributions (tail on the right) where most of the ISR samples fell within the lower half of the observed concentration ranges (Figure 6C & D). Bland–Altman plots suggested an apparent clustering of ISR failures when sample concentration was below 25 µg/ml (Figure 6A & B) [44,45]. However, a binwise comparison of ISR pass rates revealed consistently high ISR acceptance across the lower concentrations – that is, pass rate exceeded 95% when bin count was ≥15 (for AZD1061 and AZD8895, bin count was ≤15 with bins centered at 25 µg/ml; Figure 6C & D). To further evaluate ISR pass rates, the authors constructed new histograms such that the distributions were uniform – histograms had nearly equal bin counts with unequal class/bin widths (Figure 6E & F). Binwise pass rates for these uniform distributions showed consistently high pass rates across the observed concentration ranges (>95%; Figure 6E & F). Ultimately, these data reveal a reliable, reproducible assay with stable performance over time and with cumulative ISR pass rates that were well above existing guidelines for acceptance.

Taylor & Francis
Taylor & Francis Group

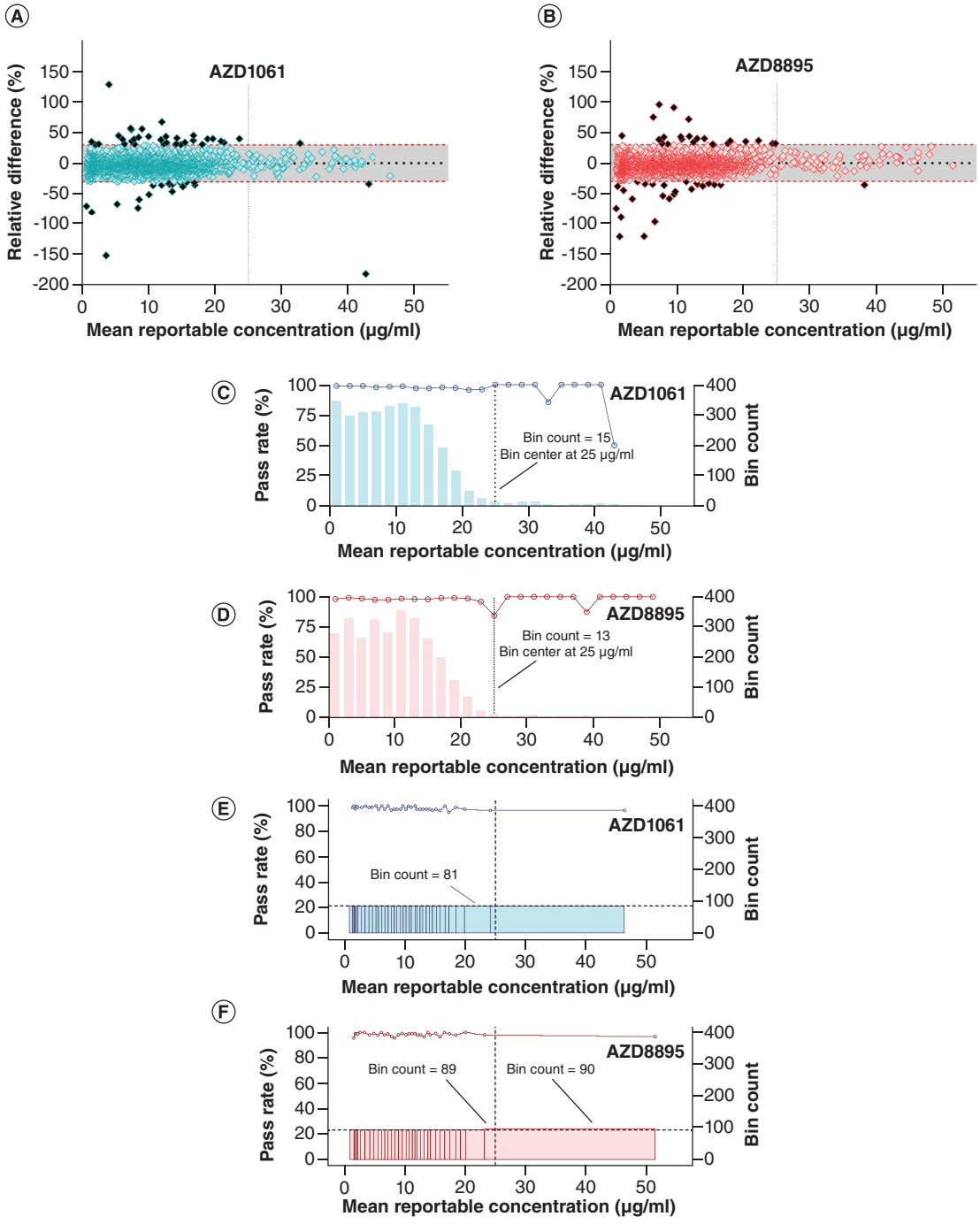

Figure 6. Concentration-dependent incurred sample reanalysis trend analysis. (A & B) Modified Bland–Altman plots of relative percent difference as a function of mean reportable concentration. Symbols with solid black fill denote data points that fall outside the acceptance limits. **(C & D)** Frequency distributions of mean reportable ISR sample concentrations: bin width fixed at 2 with first bin centered on 1.0 µg/ml. For AZD1061 and AZD8895, bin count dropped below 15 after 23.0 and 25.0 µg/ml, respectively (right y-axis). Binwise ISR pass rates for AZD1061 and AZD8895 are presented as point-to-point line graphs (right y-axis). **(E & F)** Histograms of ISR mean concentration were reconstructed as uniform frequency distributions with variable bin widths and equal, or roughly equal, bin counts (right y-axis). Binwise ISR pass rates for these reconstructed distributions are presented as point-to-point line graphs (left y-axis). **(E)** The histogram depicting AZD1061 ISR frequencies comprises 2997 observations that are split between 37 equally sized bins (81 observations each). **(F)** The histogram depicting AZD8895 incurred sample reanalysis frequencies comprises 2938 observations that are split between 33 bins. In this histogram, the first 32 bins contain 89 observations each, while the last bin contains 90 observations.

Conclusion

Within the bioanalytical community, hybrid LBA–LC–MS/MS has become more widely used and more broadly accepted. This method was developed at the height of the SARS-CoV-2 pandemic, at which time there were two fundamental challenges to the development of a traditional LBA for the absolute quantitation of these co-dosed monoclonal anti-SARS-CoV-2 antibodies (AZD1061 and AZD8895) in human serum: time and reagents. That is, development of a traditional LBA would have required critical reagents that did not yet exist and would have taken months to produce. Hybrid LBA–LC–MS/MS enabled (and expedited) assay development by precluding the need for anti-idiotype antibodies, discarding the need for separate detection antibodies, permitting the use of RBD as a capture reagent and ameliorating potential cross-reactivity issues by leveraging the selectivity of LC–MS/MS to detect unique surrogate peptides [9].

Collectively, the validation studies and assay robustness assessments reported here help establish hybrid LBA–LC–MS/MS as a mature, robust technique able to support multiple regulatory interactions. We believe these findings help establish hybrid LBA–LC–MS/MS as a robust platform for expedited assay development and provide a striking example of the technique's ability to support large-scale, long-running clinical trials. Explicitly, we reported full validation of a hybrid LBA–LC–MS/MS method for the pharmacokinetic assessment of co-dosed monoclonal anti-SARS-CoV-2 antibodies (AZD1061 and AZD8895) in human serum. Validation studies were conducted in accordance with current regulatory guidance and met regulatory expectations for method acceptance in multiple jurisdictions. Moreover, we presented findings from assay robustness assessments that included in-depth examination of QC and ISR performance trends. To our knowledge, this robustness assessment of a hybrid LBA–LC–MS/MS method is the largest and longest-running published study of its kind – that is, the reproducibility and robustness evaluations described here encompass tens of thousands of samples, from thousands of participants, in multiple clinical studies that spanned roughly a year and a half. Taken together, these validation, reproducibility and performance findings clearly demonstrate robust assay life cycle performance and reflect adequate lot, run and system consistency. Ultimately, AZD7442 (EVUSHELD) was approved in 12 countries for pre-exposure prophylaxis of COVID-19S and ten jurisdictions for treatment of mild to moderate COVID-19. Currently, the LBA–LC–MS/MS approach is being applied for the support of antibody combination therapies, such as next-generation Evusheld, AZD3152.

Ultimate validation of an assay lies in its successful application to sample analysis and the acceptable ISR that follows. In this article, we presented evidence solidifying hybrid LBA–LC–MS/MS as a robust, reliable, 'go-to' technology that can support large-scale, long-running clinical trials while meeting regulatory expectations in multiple jurisdictions. This report encompasses a remarkable number of samples, QCs and ISRs that establish confidence in the robustness of the technology and demonstrate unquestionable, long-term performance. By minimizing reliance upon specialized (critical) reagents, and by reducing assay development lead times, this technology provides options for consideration when evaluating methods to support rapid drug development. We demonstrated that hybrid LBA–LC–MS/MS is an attractive alternative to (or potential candidate for codevelopment with) traditional LBAs, especially when challenges arise during development of LBAs. Over the next 5–10 years, we anticipate that the bioanalytical community will continue to be challenged with ever more complex biotherapeutic modalities. As such, we believe that methods using this technology can substantially accelerate the drug development time line in bringing the right drug candidates to the patients.

Summary points

- AZD7442 is comprised of co-dosed, long-acting IgG monoclonal antibodies: cilgavimab (AZD1061) and tixagevimab (AZD8895).
- The authors fully validated a hybrid ligand-binding assay LC–MS/MS method for absolute quantitation of these co-dosed monoclonal antibodies.
- Validation study results met all current US FDA regulatory guidelines for method acceptance.
- The method was used to support multiple clinical trials; three were discussed here: TACKLE, STORM CHASER and PROVENT.
- Cumulative incurred sample reanalysis pass rates for AZD1061 and AZD8895 were ≥98%.
- To the authors' knowledge, the method robustness assessments presented here are the most expansive published evaluations of their kind of a hybrid ligand-binding assay LC–MS/MS method. The method robustness assessment spanned 17 months, from April 2021 through October 2022 and included at least 720 analytical runs, roughly 30,000 samples and ~3000 incurred sample reanalyses per analyte.

Taylor & Francis
Taylor & Francis Group

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/suppl/10.4155/bio-2023-0225

Acknowledgments

The authors were fortunate to work with many capable, dedicated people and thank all members of the bioanalytical teams at AstraZeneca and PPD CSD R&D (VA, USA). For her specific administrative contributions to this project, the authors recognize Diane MZ Lebarbenchon. Diane, a vital member of the PPD administrative team, provided critical clerical support and tireless work throughout the development of these assays and timely editorial support during the writing of this manuscript.

Financial disclosure

This study was funded by AstraZeneca and the Defense Advanced Research Projects Agency under HR0011-18-3-0001 (Distribution Statement A. Approved for Public Release. Distribution Unlimited). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. AstraZeneca provided funding to PPD for the conduct of this study.

Competing interests disclosure

Authors Y Huang, CC Wang and AI Rosenbaum performed work within this manuscript while employed by AstraZeneca. The authors' employment and stock investments in AstraZeneca do not affect the authenticity and objectivity of the experimental result detailed in this manuscript. Authors MS Woolf, SM Naser, AM Wheeler, WR Mylott and E Ma performed work within this manuscript while employed by PPD, a part of Thermo Fisher Scientific. The authors' employment and stock investments in Thermo Fisher Scientific do not affect the authenticity and objectivity of the experimental result detailed in this manuscript. Responsibility for opinions, conclusions and data interpretation lies with the authors and should not be interpreted as representing the official views or policies of the Department of Defense or the US government. The authors have no other competing interests or relevant affiliations with any organization or entity with the subject matter or materials discussed in the manuscript apart from those disclosed.

Writing disclosure

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

This study was conducted in accordance with principles of the Declaration of Helsinki and International Conference on Harmonisation Guidance for Good Clinical Practice. Independent ethics committee approval was obtained.

Data sharing statement

All relevant data required to replicate the study's findings are within the paper and the supplemental information. Raw data can be obtained in accordance with AstraZeneca's data sharing policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Mullard A. FDA approves 100th monoclonal antibody product. *Nat. Rev. Drug Discov.* 20(7), 491–495 (2021).
- **This retrospective article describes the emergence and advancement of therapeutic monoclonal antibody products.**
2. Todd PA, Brogden RN. Muromonab CD3. *Drugs* 37(6), 871–899 (1989).
3. Singh S, Tank NK, Dwiwedi P *et al.* Monoclonal antibodies: a review. *Curr. Clin. Pharmacol.* 13(2), 85–99 (2018).
4. Ecker DM, Jones SD, Levine HL. The therapeutic monoclonal antibody market. *MAbs* 7(1), 9–14 (2015).
5. Lindsley CW. Predictions and statistics for the best-selling drugs globally and in the United States in 2018 and a look forward to 2024 projections. *ACS Chemical Neuroscience* 10(3), 1115–1115 (2019).
6. Ezan E, Bitsch F. Critical comparison of MS and immunoassays for the bioanalysis of therapeutic antibodies. *Bioanalysis* 1(8), 1375–1388 (2009).
7. Zhang YJ, Olah TV, Zeng J. The integration of ligand binding and LC–MS-based assays into bioanalytical strategies for protein analysis. *Bioanalysis* 6(13), 1827–1841 (2014).

8. Ismaiel OA, Mylott WR Jr, Jenkins RG. Do we have a mature LC–MS/MS methodology for therapeutic monoclonal antibody bioanalysis? *Bioanalysis* 9(17), 1289–1292 (2017).

• **This commentary discusses different LC–MS/MS approaches for robust bioanalysis of biotherapeutic monoclonal antibodies; topics include signature surrogate and universal surrogate peptides, internal standard selection, bioanalysis of antibody-drug conjugate (ADC) total antibody and acceptance criteria for LC–MS/MS methods of therapeutic proteins.**

9. Mu R, Huang Y, Bouquet J *et al.* Multiplex hybrid antigen-capture LC–MRM quantification in sera and nasal lining fluid of AZD7442, a SARS-CoV-2-targeting antibody combination. *Anal. Chem.* doi:10.1021/acs.analchem.2c01320 (2022) (Epub ahead of print).

•• **This preclinical report established hybrid ligand-binding assay–LC–MS/MS as a highly sensitive and reproducible platform for the quantification of therapeutic antibodies. These studies also demonstrate the utility of hybrid ligand binding-assay–LC–MS/MS for the bioanalysis of specialized matrices, such as nasal lining fluid.**

10. Desilva B. Immunoaffinity-coupled MS: best of both technologies. *Bioanalysis* 8(15), 1543–1544 (2016).

11. Dubois M, Fenaille F, Clement G *et al.* Immunopurification and mass spectrometric quantification of the active form of a chimeric therapeutic antibody in human serum. *Anal. Chem.* 80(5), 1737–1745 (2008).

12. Ezan E, Dubois M, Becher F. Bioanalysis of recombinant proteins and antibodies by mass spectrometry. *Analyst* 134(5), 825–834 (2009).

13. Ackermann BL, Berna MJ. Coupling immunoaffinity techniques with MS for quantitative analysis of low-abundance protein biomarkers. *Expert Rev. Proteomics* 4(2), 175–186 (2007).

14. Stevenson L, Garofolo F, Desilva B *et al.* 2013 white paper on recent issues in bioanalysis: 'hybrid' – the best of LBA and LCMS. *Bioanalysis* 5(23), 2903–2918 (2013).

15. Ackermann B, Neubert H, Hughes N *et al.* 2015 white paper on recent issues in bioanalysis: focus on new technologies and biomarkers (part 2 – hybrid LBA/LCMS and input from regulatory agencies). *Bioanalysis* 7(23), 3019–3034 (2015).

•• **This paper encompasses industry recommendations that emerged from the 9th Workshop on Recent Issues in Bioanalysis. Part 2 includes recommendations for hybrid ligand-binding assay/LC–MS, with input from regulatory agencies.**

16. Dufield D, Neubert H, Garofolo F *et al.* 2014 white paper on recent issues in bioanalysis: a full immersion in bioanalysis (part 2 – hybrid LBA/LCMS, ELN & regulatory agencies' input). *Bioanalysis* 6(23), 3237–3249 (2014).

17. Jenkins R, Duggan JX, Aubry A-F *et al.* Recommendations for validation of LC–MS/MS bioanalytical methods for protein biotherapeutics. *AAPS J.* 17(1), 1–16 (2015).

•• **This paper presents North American bioanalytical industry recommendations regarding the validation and application of LC–MS/MS methods for bioanalysis of protein biotherapeutics in regulated studies.**

18. Ramagiri S, Moore I. Hybridizing LBA with LC–MS/MS: the new norm for biologics quantification. 8(6), 483–486 (2016).

19. Ackermann BL. Immunoaffinity MS: adding increased value through hybrid methods. 8(15), 1535–1537 (2016).

20. Kaur S, Bateman KP, Glick J *et al.* IQ consortium perspective: complementary LBA and LC–MS in protein therapeutics bioanalysis and biotransformation assessment. *Bioanalysis* 12(4), 257–270 (2020).

21. Yuan M, Ismaiel OA, Mylott W. Hybrid ligand binding immunoaffinity-liquid chromatography/mass spectrometry for biotherapeutics and biomarker quantitation: how to develop a hybrid LBA–LC–MS/MS method for a protein. *Rev. Separation Sci*. 1(1), 47–55 (2019).

22. Saxena A, Patel A, Mehta P. Comparison and recent updates of LBA and LC–MS/MS methods for bio-analysis of therapeutic monoclonal antibodies. *Acta Sci. Pharm. Sci.* 5(5), 13–25 (2021).

23. Xu K, Liu L, Maia M *et al.* A multiplexed hybrid LC–MS/MS pharmacokinetic assay to measure two co-administered monoclonal antibodies in a clinical study. *Bioanalysis* 6(13), 1781–1794 (2014).

24. Kellie JF, Kehler JR, Mencken TJ, Snell RJ, Hottenstein CS. A whole-molecule immunocapture LC–MS approach for the *in vivo* quantitation of biotherapeutics. *Bioanalysis* 8(20), 2103–2114 (2016).

25. Kaur S, Liu L, Cortes DF *et al.* Validation of a biotherapeutic immunoaffinity–LC–MS/MS assay in monkey serum: 'plug-and-play' across seven molecules. *Bioanalysis* 8(15), 1565–1577 (2016).

26. Tilly H, Morschhauser F, Sehn LH *et al.* Polatuzumab vedotin in previously untreated diffuse large B-cell lymphoma. *N. Engl. J. Med.* 386(4), 351–363 (2022).

27. R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing. (2018).

28. Team R. RStudio: integrated development for R. RStudio, PBC. (2020).

29. U.S. Food & Drug Administration (FDA). *Bioanalytical Method Development and Validation: Guidance for Industry.* Silver Spring, MD (2018).

30. U.S. Department of Health and Human Services, Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), & Center for Veterinary Medicine (CVM). *Bioanalytical Method Validation.* Washington, D.C. (2001).

31. A phase III randomized, double-blind, placebo-controlled, multi-center study in adults to determine the safety and efficacy of AZD7442, a combination product of two monoclonal antibodies (AZD8895 and AZD1061), for post-exposure prophylaxis of COVID-19. (STORM CHASER). https://clinicaltrials.gov/study/NCT04625972

Taylor & Francis
Taylor & Francis Group

32. A phase III randomized, double-blind, placebo-controlled, multicenter study to determine the safety and efficacy of AZD7442 for the treatment of COVID-19 in non-hospitalized adults. (TACKLE). https://clinicaltrials.gov/study/NCT04723394

33. AZD7442 PROVENT phase III prophylaxis trial met primary endpoint in preventing COVID-19 (2021).

34. *Bioanalytical Method Validation and Study Sample Analysis M10.* ICH, Geneva, Switzerland (2022).

35. Faria M, Peay M, Lam B *et al.* 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* 8(1), 11 (2019).

• **This report describes the development, validation and application of three novel, multiplex ligand bind assay–LC–MS/MS bioanalytical methods for the measurement of five analytes from MEDI4276 (an antibody-body-drug conjugate [ADC]) in human plasma: total antibody, conjugated payload (total ADC), conjugated payload metabolite, unconjugated payload and unconjugated payload metabolite.**

36. Huang Y, Del Nagro CJ, Balic K *et al.* Multifaceted bioanalytical methods for the comprehensive pharmacokinetic and catabolic assessment of MEDI3726, an anti-prostate-specific membrane antigen pyrrolobenzodiazepine antibody–drug conjugate. *Anal. Chem.* 92(16), 11135–11144 (2020).

•• **This article is an excellent primer on hybrid ligand-binding assay–LC–MS/MS as an analytical tool and provides key insights into how this platform can be used for a variety of pharmacokinetic studies. This paper will prove especially useful to those who are unfamiliar with this type of bioanalysis.**

37. Levin MJ, Ustianowski A, De Wit S *et al.* Intramuscular AZD7442 (tixagevimab–cilgavimab) for prevention of COVID-19. *N. Engl. J. Med.* 386(23), 2188–2200 (2022).

38. Levin MJ, Ustianowski A, Thomas S *et al.* AZD7442 (tixagevimab/cilgavimab) for post-exposure prophylaxis of symptomatic coronavirus disease 2019. *Clin. Infect. Dis.* 76(7), 1247–1256 (2023).

39. Montgomery H, Hobbs FR, Padilla F *et al.* Efficacy and safety of intramuscular administration of tixagevimab–cilgavimab for early outpatient treatment of COVID-19 (TACKLE): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet Respir. Med.* 10(10), 985–996 (2022).

40. Azadeh M, Sondag P, Wang Y, Raines M, Sailstad J. Quality controls in ligand binding assays: recommendations and best practices for preparation, qualification, maintenance of lot to lot consistency, and prevention of assay drift. *AAPS J.* 21(5), 1–14 (2019).

41. Makowski N, Ciplea AM, Ali M, Burdman I, Bartel A, Burckhardt BB. A comprehensive quality control system suitable for academic research: application in a pediatric study. *Bioanalysis* 12(5), 319–333 (2020).

42. European Medicines Agency (EMA), Amsterdam. *Guideline on Bioanalytical Method Validation* (Rev. 1 Corr. 2) (2011).

43. Kall MA, Michi M, Van Der Strate B, Freisleben A, Stoellner D, Timmerman P. Incurred sample reproducibility: 10 years of experiences: views and recommendations from the European Bioanalysis Forum. *Bioanalysis* 10(21), 1723–1732 (2018).

•• **This white paper presents and discusses the results of three surveys that were conducted in 2016 and 2017. The views expressed in the paper highlight European pharmaceutical industry and contract research organization experiences with and recommendations for incurred sample reanalysis.**

44. Rudzki PJ, Biecek P, Kaza M. Comprehensive graphical presentation of data from incurred sample reanalysis. *Bioanalysis* 9(12), 947–956 (2017).

45. Bland JM, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 327(8476), 307–310 (1986).