MEDPACE
BIOANALYTICAL LABORATORIES

BIOANALYTICAL METHOD VALIDATION PROTOCOL

VALIDATION OF AN LC-MS/MS METHOD FOR THE QUANTITATIVE DETERMINATION OF LB-102 AND LB-101 IN HUMAN K_2EDTA PLASMA

MBL Study Number: MBL19314

Testing Facility: Medpace Bioanalytical Laboratories
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USA

Sponsor: LB Pharmaceuticals, Inc.
575 Madison Avenue
New York, NY 10022

Proposed Start Date : 02-Jan-2020
Estimated Completion Date : 05-Mar-2020
Estimated Draft Report Date : 19-Mar-2020

REVISION HISTORY

VERSION DATE	REVISION HISTORY DETAILS
02-Jan-2020	First issuance of the protocol for study MBL19314

SIGNATURES

Study Protocol Approval:

Shuming Yang — 02 Jan 2020

Shuming Yang, Ph.D.
Study Director
Medpace Bioanalytical Laboratories — Date

02-Jan.-2020

Yong-Xi Li, Ph.D.
Executive Director
Medpace Bioanalytical Laboratories — Date

12/23/19

Zach Prensky,
CEO
LB Pharmaceuticals, Inc. — Date

QA Review By:

02 Jan 2020

Jean Miller, BS
Senior QA Auditor
Medpace, Inc. — Date

LIST OF ABBREVIATIONS

The following abbreviations may be used:

µg	Microgram(s)
µL	Microliter(s)
CV	Coefficient of Variation
DQC	Dilution Quality Control Sample
DMSO	Dimethyl Sulfoxide
EDTA	Ethylene Diamine Tetraacetic Acid
FDA	Food and Drug Administration (US)
GLP	Good Laboratory Practice
HPLC	High Performance Liquid Chromatography
HQC	High QC
hr	Hour
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IS	Internal Standard
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LIMS	Laboratory Information Management System
LLOQ	Lower Limit of Quantitation
LQC	Low QC
M	Molar
MBL	Medpace Bioanalytical Laboratories
mg	Milligram(s)
mL	Milliliter(s)
mm	Millimeter(s)
mM	Millimolar
MQC	Mid QC
MS	Mass Spectrometry
ng	Nanogram(s)
No.	Number
pg	Picogram(s)
QA/QAU	Quality Assurance/Quality Assurance Unit
QC	Quality Control
RE	Relative Error
S.D.	Standard Deviation
SOP	Standard Operating Procedure
Std/STD	Standard
ULOQ	Upper Limit of Quantitation

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1.0 INTRODUCTION

This validation will be conducted in human K_2EDTA plasma following this protocol and applicable Medpace Bioanalytical Laboratories' (MBL) SOPs, in particular, US-BL-39.

2.0 OBJECTIVE

The objective of this study is to validate a method to quantitate LB-102 and LB-101 in human K_2EDTA plasma to support clinical analysis. The validation will assess the following parameters:

- Standard Weighing Comparison
- Calibration Curve Linearity
- Precision and Accuracy
- Selectivity
- Carryover
- Dilution Integrity and Linearity
- Sample Processing Recovery
- Matrix Effect
- Hemolysis Effect
- Freeze/Thaw Stability
- Bench-top Stability
- Autosampler Stability
- Extract Storage Stability
- Long-term Frozen Sample Storage Stability
- Stock Solution and Working solution Stability
- Batch Size Verification
- Ruggedness
- System Suitability

3.0 ROLES AND RESPONSIBILITIES

3.1 Study Director

The study director is appointed by Medpace Bioanalytical Laboratories and has overall responsibility for conduct of this validation. The study director shall ensure all validation work performed will be conducted in a manner consistent with the Good Laboratory Practice (GLP) principles outlined in the U.S. FDA Title 21 CFR Part 58 regulations and MBL SOPs.

3.2 Quality Assurance Audit (QAU)

The Medpace QAU will audit this bioanalytical validation study as prescribed in the GLP regulations and according to relevant Medpace Quality Assurance (QA) SOPs. All the documents, procedures, materials and instruments etc., pertinent to the validation, including but not limited to, reference standard receipt and storage, stock and working solution preparation, calibration standard and quality control (QC) sample preparation, sample preparation and LC-MS/MS procedure, may be subject to QA audit. All analytical data and the validation report will be audited by the QAU according to relevant Medpace QA SOPs.

3.3 Sponsor Responsibilities

The sponsor is responsible for providing reference materials of known strength, purity, expiration date, and storage conditions. Reference materials may also be obtained from a reliable, preferably commercial, source.

Upon completion of the experiments in the study, a QC reviewed and/or QA audited electronic draft report will be sent to the sponsor. The sponsor should review the report and provide comments within four weeks. The study director will make appropriate changes to finalize the report. The report signature pages should be completed and returned within four weeks.

4.0 EQUIPMENT, REFERENCE STANDARDS AND MATRICES

4.1 LC-MS/MS System

The system will consist of a triple quad 5500 or equally sensitive MS system coupled with Shimadzu or Waters HPLC system. Full details of the LC-MS/MS conditions are provided in the analytical method.

4.2 Reference Standards

The reference standards, LB-102 and LB-101 and internal standards, LB-102-d5 and Amisulpride-d5 (LB-101-d5), will be provided by the sponsor or obtained from commercially available vendors. All reference standards will be stored at appropriate storage conditions. Certificates of Analysis, lot and/or batch numbers, expiration (or re-evaluation/re-test) dates, storage conditions, stability information (if available), and purity information will be maintained in notebook or study records in accordance with all applicable Medpace Bioanalytical Laboratories SOPs.

4.3 Biological Matrices

Human K_2EDTA plasma and human whole blood K_2EDTA will be obtained from reliable sources, suitably stored, and the source recorded.

5.0 SOLUTION PREPARATION

5.1 Stock Solutions

Reference standard stock solutions will be prepared at 1.0 mg/mL in dimethyl sulfoxide (DMSO), verified, and stored at -20°C.

5.2 Calibration Standards

Calibration standards will be prepared by spiking the analytes into human plasma. A calibration curve will consist of at least six non-zero concentrations over the range 1.00 ng/mL to 1000 ng/mL. The calibration standards will be injected in duplicate, one at the beginning of the batch and one at the end of the run sequence, thereby bracketing all samples. Calibration standards will be freshly prepared for all intra-/ inter-assay precision and accuracy runs and matrix stability assessment runs.

5.3 Quality Control Samples

QC samples will be prepared from separate stock solutions from those used for preparation of the calibration standards, unless stock solution equivalency is demonstrated. QC samples will be prepared by directly spiking analyte solution into an appropriate volume of blank matrix, and at four different concentrations: 1.00 ng/mL (LLOQ), 3.00 ng/mL (LQC), 30.0 ng/mL (MQC), and 750 ng/mL (HQC). QC should be prepared at each level and stored for subsequent assessment for stability. All stability QC samples will be stored at -20°C and/or -70°C. Separate sets of QC samples will be freshly extracted for all intra-/ inter-assay precision and accuracy batches.

The precision and accuracy of the method will be determined by analyzing QC samples in replicates of six. All QC samples at each concentration level must be used for statistical calculations unless there is a valid reason to discount any individual sample. Two-thirds of all QC samples and half at each concentration level should be within 15% (20% for LLOQ) of the nominal concentration.

6.0 EXPERIMENTS

A typical validation run will contain at least a complete calibration curve, three levels of QC samples, i.e., the analytical QCs (LQC, MQC and HQC), in duplicate, to verify assay performance, and at least one blank sample and a blank sample with internal standard in addition to other samples necessary to carry out the validation.

Note that if the initial stability assessment does not meet acceptance criteria, the stability can be repeated at the same stability condition of equal or less durations/cycles and interim stability points may also be evaluated.

6.1 Standard Weighing Comparison

In general, stock solutions for standards and QC samples will be prepared from separate weighings. A single stock solution may be used for both the standards and QCs if equivalency is demonstrated between the two independent weighings. Internal standard will be prepared from a single weighing and no weighing comparison will be performed.

Stock solutions from separate weighings will be diluted to the same concentration level within the calibration curve range, injected in at least triplicate, and analyzed for comparison.

6.2 Calibration Curve Linearity

The standard curve fitting method should be determined by applying the simplest model that adequately describes the concentration-response relationship. Linear fits with a weighting factor of $1/x^2$ should be suitable for this method. The same fitting method should be applied to all validation runs and future study sample analysis runs.

6.3 Precision and Accuracy

The intra-assay precision and accuracy will be measured from six replicate QC samples at LLOQ, LQC, MQC and HQC concentrations over the range studied. The inter-assay precision and accuracy is ascertained by analyzing six replicates of the LLOQ, LQC, MQC and HQC samples in at least three separate analytical runs.

Note that for any validation runs that do not include accuracy and precision (*e.g.*, for stability assessment), duplicate injection standard curves and at least duplicate QC samples at LQC, MQC

and HQC concentrations, as analytical QCs, will be used to determine the acceptability of these runs.

6.4 Selectivity

The selectivity of the assay for LB-102 and LB-101 will be assessed in human plasma using at least six individual lots of normal plasma. An additional lot of lipemic plasma and hemolyzed plasma will also be assessed. The eight individual lots will be evaluated using double blanks (blank matrices only) and blank spiked with LB-102 and LB-101 at the LLOQ level.

6.5 Carryover

Carryover will be assessed in all validation runs by injecting a blank sample after the first injection of the highest standard in the run or high QC sample. In the event that a measurable peak is detected in the blank sample, it should not exceed 20% of the mean signal of LLOQ standard samples in the analyte channel, and not exceed 5% of the mean signal of the IS response of the LLOQ standards. If carryover above the specification is observed, additional measures (e.g., injection of blank samples following study samples that are expected to have a high concentration, or use of an additional autosampler wash or other wash solution) will be tested and assessed. If additional measures are needed in case of persistent carryover, they will be documented in the raw data and/or in the method. Acceptance/rejection of batches or samples due to carryover above the specification will be discussed in the final report and reflected in the application of the method.

6.6 Dilution Integrity and Linearity

Dilution QC will be prepared at a concentration approximately 5 times the upper limit of quantitation (ULOQ). This QC will be diluted at least 10-fold and 100-fold with matrix, so that the concentrations fall within the range of the curve and will be analyzed within a validation batch.

6.7 Sample Processing Recovery

Recovery through the extraction process will be evaluated by comparing response from extracted samples in at least triplicate from LQC, MQC and HQC samples to those of post-spiked blank extracted samples at the same nominal concentrations.

6.8 Matrix Effect

The matrix effect of human plasma on the quantitation of LB-102 and LB-101 will be assessed using six individual lots of regular plasma plus one additional lot of lipemic plasma. The individual

lots of plasma will be extracted in triplicate at the LQC and HQC levels. The mean will be calculated for each individual lot and compared to nominal concentrations.

6.9 Hemolysis Effect

The effects of hemolysis of blood on LB-102 and LB-101 will be determined in a regular plasma fortified with 2% volume of completely hemolyzed whole blood. At least 5 replicates of samples at the LQC and HQC concentrations will be prepared in this matrix, extracted and quantified to assess the effect of the hemolysis.

6.10 Freeze/Thaw Stability

The effects of subjecting samples to at least three cycles of freezing and thawing (under -20°C and -70°C storage conditions) will be determined. Samples at LQC and HQC concentrations will be stored for at least 24 hours at -20°C or -70°C and thaw unassisted at ambient temperature. Once completely thawed, the samples will be refrozen for at least 12 hours under the same conditions; the thawing process will be the same as cycle 1.

6.11 Bench-top Stability

The stability of samples stored at ambient will be determined for at least six hours. At least three replicate QC samples will be extracted at the LQC and HQC concentrations after storage at ambient and quantified against freshly prepared calibration standards and QC samples.

6.12 Autosampler Stability

Calibrators and QCs from a previously accepted batch will be reinjected after storage at 4°C for at least 24 hours to demonstrate autosampler stability.

6.13 Extract Storage Stability

The stability of extracted samples stored at 4°C will be determined for at least 24 hours. At least three replicate LQC and HQC samples will be quantified after storage against freshly prepared calibration standards and QC samples.

6.14 Frozen Sample Long-Term Storage Stability

The stability of matrix samples stored at -20°C and -70°C will be determined for ~2 months (or longer). At least three replicate QC samples at the LQC and HQC concentrations after storage will be extracted and quantified against freshly prepared calibration standards and QC samples.

6.15 Stock Solution and Working Solution Stability

Stock solution stability of LB-102 and LB-101 will be determined for at least six hours at ambient temperature to demonstrate stability of the stock solutions for the time they may be left at ambient temperature during use.

Stock solutions stability of LB-102 and LB-101 will be determined for ~2 months or longer for stocks stored at -20°C. Stock solution stability is determined for the time they may be stored for validation and sample analysis. At least 3 replicates of freshly prepared stocks will be compared with stock solutions stored at ambient or -20°C.

Working solutions stability of LB-102 and LB-101 will be determined for ~2 months or longer for working solutions stored at -20°C. Working solution stability is determined for the time they may be stored for validation and sample analysis. At least 3 replicates of freshly prepared working solution samples will be compared with working solutions samples stored at ambient or -20°C.

6.16 Batch Size

At least one validation run will be prepared with the anticipated maximum number of injections for sample analysis batches to demonstrate the performance of the method with acceptable standards and QCs.

6.17 Ruggedness

A passing validation run will be reinjected in a different LC-MS/MS system or using a different HPLC column. Ruggedness is demonstrated if the run meets the acceptance criteria.

7.0 ACCEPTANCE CRITERIA

Acceptance criteria are listed below and are based on Medpace SOP US-BL-39. Any differences in the required experiments and/or acceptance criteria in the SOP due to the nature of the method or the extent of validation needed are indicated in the table below (or may be added and/or modified by amendment, if necessary).

Validation Items	Minimum Experiment	Acceptance Criteria
Standard weighing comparison:	2 separate weighings, ≥3 replicates per weighing	The mean area counts (or area ratio of internal standard included) of stock solutions from separate weighings should be within ±7% of each other

Validation Items	Minimum Experiment	Acceptance Criteria
Calibration curve linearity:	≥3 runs (≥6 non-zero STDs)	75% of the STDs (including at least one at the LLOQ and one at the ULOQ) within ±15% of nominal concentrations (±20% at LLOQ)
Intra-assay precision and accuracy:	6 replicates per QC level	67% of all QCs and 50% at each level and overall mean: RE within ±15% (±20%LLOQ) CV ≤15% (≤20%LLOQ)
Inter-assay precision and accuracy:	QCs in at least 3 runs	RE within ±15% (±20%LLOQ) CV ≤15% (≤20%LLOQ)
Selectivity:	A) Blank Matrix: Six lots of normal matrix, one lot of lipemic matrix and one lot of hemolyzed matrix will be extracted without the analyte and/or IS. B) Analyte at LLOQ will be analyzed in eight lots of matrix	A) Analyte: Interference ≤20% of LLOQ response IS: Interference ≤5% of IS response B) The RE of measured individual concentrations at the LLOQ should be ±20% in at least 80% (about 7 of 8) of the individual lots
Carryover:	Double blank, reagent blank or mobile phase blank injected after the highest STD or QC	Analyte: Carryover ≤20% of mean peak area of LLOQ STD samples IS: Carryover ≤5% of mean peak area of IS in LLOQ STD samples
Dilution integrity and linearity:	10-fold and 100-fold dilution at 5000 ng/mL (n≥5)	RE within ±15% CV ≤15%
Sample processing recovery:	Extracted LQC, MQC & HQC (n≥3) vs. post-spiked blank extract at the corresponding LQC, MQC & HQC concentrations (n≥3)	There are no formal acceptance criteria for recovery. Recovery of the analyte need not be 100%, but the extent of recovery should be consistent within and across all QC levels. Moreover, precision for each analyte and IS must be ≤15% CV at every QC level.
Matrix effect:	6 lots of normal plasma and 1 lot of lipemic plasma in triplicate at LQC & HQC levels	Mean of replicates for individual lots and overall mean of all lots RE within ±15% CV ≤15%
Hemolysis effect:	A set of LQC & HQC (n≥5) in 2% hemolyzed matrix	RE within ±15% CV ≤15%
Freeze/Thaw stability:	A set of LQC & HQC (n≥3) ≥ 3 cycles at -20°C and -70°C	RE within ±15% CV ≤15%
Bench-top stability:	A set of LQC & HQC (n≥3) stored at ambient for ≥ 6 hrs	RE within ±15% CV ≤15%
Autosampler stability:	One run with at least 3 LQC & HQC replicates, reinjected after ≥24 hrs stored	Calibration and QC samples must meet acceptance criteria

Validation Items	Minimum Experiment	Acceptance Criteria
Extract storage stability:	A set of LQC & HQC (n≥3) stored at 4°C for ≥ 24 hrs	RE within ±15% CV ≤15%
Frozen sample long-term storage stability:	A set of LQC & HQC (n≥3) stored at -20°C and -70°C for ~2 month2 or longer	RE within ±15% CV ≤15%
Stock solution and working solution stability:	A) Stock stored ambient for at least 6 hrs compared to freshly prepared stocks(s) (n≥3) B) Stock(s) stored at -20°C for ~2 months or longer compared to freshly prepared stock(s) (n≥3) C) Working solutions (W-Std-1 and W-Std-8) used and stored at -20°C for ~2 months or longer compared to freshly prepared working(s) (n≥3)	Mean peak area (or area ratio against internal standard) of ambient/frozen aliquots within ±15% of fresh
Batch size:	A run with a large number of samples expected for sample analysis	Calibration and QC samples must meet acceptance criteria
Ruggedness:	A passing run reinjected using different column or instrument type	Calibration and QC samples must meet acceptance criteria

8.0 DATA ACQUISITION AND PROCESSING

The current version of Sciex Analyst software (Applied Biosystems) will be used for data acquisition and integration of chromatograms. Following integration, the result tables from Analyst will be imported into Watson LIMS (Thermo Fisher Scientific, Inc.) where regression will be performed. Statistical parameters such as means, standard deviations, coefficients of variation, and relative error will be exported from Watson LIMS and/or calculated using Microsoft Excel.

Measured concentrations will be reported to three significant figures. The standard deviation (S.D.) will be reported to the same significant figures as the mean concentration. All percentage data will be reported with one decimal place.

9.0 REPORT

The results from validation experiments will be compiled into a validation report. The validation report will be in Medpace report format. A draft validation report will be sent to the sponsor for review and comments, and received comments, if applicable, will be incorporated in the final report by the study director. The final report will be audited by the QAU prior to issuance. After the report is issued, the sponsor will receive a PDF version of the original final report. Any additional stability data generated after the initial validation report will be included in a report addendum or amendment.

10.0 METHOD

The bioanalytical method MBL19314-M0.1 is in draft form and is subject to change pending validation results. Minor modifications that do not require additional validation work may be made with proper justification during the course of the validation without protocol amendment. Changes will be clearly documented in the raw data. A protocol amendment is required for method modifications that require additional validation work. The validated method will be issued as a separate document as version 1.0 prior to sample analysis.

11.0 MAINTAINING RECORDS AND ARCHIVING

Records to be maintained by Medpace Bioanalytical Laboratories will include, but not be limited to, the following, where appropriate:

- Signed validation study protocol and amendments
- Deviations from the study protocol or SOPs
- Calibration standard and quality control sample preparation and usage records, location and storage conditions of QC samples
- Control matrix information (including: receipt and storage records)
- Reference and internal standard information (including: certificates of analysis, receipt, usage, and storage records)
- Reagent preparation records
- Batch data (including: chromatograms, Analyst, and Watson printouts)
- Study Notebook
- Other documents as needed (including: spreadsheets, report tables, and regression analysis data)
- Correspondence
- Final report

- Method
- Report references

Upon archiving, all study data including the report, study file, laboratory notebooks, instrument printouts, electronic data and any other study records will be stored in a Medpace designated archive facility, Access Information Protected/Retrievex (690 E Crescentville Road, Cincinnati, OH 45246) for a maximum of three years. The study director or designee will contact the sponsor for the disposition of any study-specific material meeting the maximum retention period.