Method Validation for the Evaluation of Plasma Protein Binding of LGX818 and MEK162 by Rapid Equilibrium Dialysis and LC-MS/MS

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Introduction

Drug efficacy seems to be closely related to unbound drug concentrations at the site of action. The determination of plasma unbound concentrations/fractions (Fu) of two approved anti-cancer small molecules LGX818and MEK162 (selective BRAF and MEK inhibitors, respectively) is of crucial importance to have a deep understanding of pharmacokinetic (PK) and pharmacodynamic (PD) profiles of the drugs in various targeting patient populations and for the optimization of dosing regimens to ensure adequate efficacy and safety. In the current study, a method was developed for simultaneously determining the unbound fraction (Fu) of LGX818 and MEK162 in human plasma by using rapid equilibrium dialysis (RED) for the separation of the bound and unbound drug, and LC–MS/MS for subsequent quantification.

The rapid equilibrium dialysis (plasma vs phosphate buffer solution (PBS)) of a mixture of LGX818 and MEK162 is established with the determination of equilibrium. Percentage (%) of fraction unbound (Fu) of the drugs are obtained with the quantification (by LC-MS/MS) of the amount of drugs in plasma and the amount of unbound drugs in the receiver reservoir of the RED device system (Thermo Scientific, RED re-useable base plate. For the aforementioned quantifications, an LC-MS/MS method was validated for the determination of LGX818 and MEK162 simultaneously in human plasma (K_2EDTA) and PBS (1:1, v/v) mixture. The Fu was evaluated at two concentration levels (Low PPB QC concentration and High PPB QC concentration). Incubation stability (with regards to equilibrium dialysis) of LGX818 and MEK162 in plasma and in PBS was studied, by using a validated LC-MS/MS method.

Methodology

Summary of Methodology for Plasma Protein Binding Evaluation	
Method	Rapid Equilibrium Dialysis
Matrix	Plasma
Dialysis Buffer	Phosphate Buffer Solution (PBS)
Incubation	37℃，5% Carbon Dioxide
Incubation Time	7 Hours
Concentration Evaluated	LGX818: LQC (200 ng/mL), HQC (700 ng/mL) MEK162: LQC (100 ng/mL), HQC (350 ng/mL)
Replicates	6 at each QC level
Detection/Quantification	LC-MS/MS
Evaluation Aspects	Fu CV；Incubation Stability；Incubation Recovery

- Fraction unbound (%Fu) and %Protein Binding Rate

$$\%Fu = \frac{[C]_{7h}\ of\ R\ side}{[C]_{7h}\ of\ D\ side} \times 100\% \quad (1) \qquad \%Protein\ Binding = 100\% - \%Fu \quad (2)$$

- %Recovery of the plasma protein binding experiment

$$\%Recovery = \frac{[C]_{7h}\ of\ R\ side \times 350\ \mu L + [C]_{7h}\ of\ D\ side \times 200\ \mu L}{[C]_{Nominal} \times 200\ \mu L} \times 100\% \quad (3)$$

Experimental

- Rapid Equilibrium Dialysis

RED base plate, Catalog No. 89811 / RED insert, Catalog No. 89809 / MWCO 8000 Da

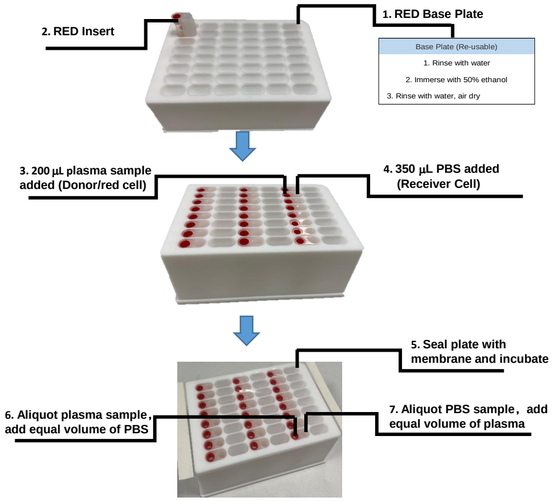

Figure 1. Experimental Steps of Rapid Dialysis Equilibrium

- LC-MS/MS Analysis of Samples Collected from RED

Compounds	LGX818, MEK162	
Matrix	Plasma/PBS (1:1, v/v)	
Linear Range	LGX818: 1.00 - 1000 ng/mL; MEK162: 0.500 - 500 ng/mL	
Sample Preparation	Protein Precipitation (PPT) Sample aliquot：50 µL Plasma/PBS (1:1, v/v)	
HPLC	HPLC：Shimadzu Nexera UHPLC Column：Waters ACQUITY UPLC BEH C18 Column Mobile Phases：5mM NH_4FA in water; 0.2% FA in ACN Elution：Gradient Run time：4 min	
Mass Spectrometry	MS：SCIEX 6500+ Scan Mode：Positive，MRM LGX818 and MEK162 monitored by their respective SIL IS	
Method Validation	Selectivity and Specificity Precision and Accuracy Sensitivity Extraction Recovery and Matrix Effect	Linearity Carry Over Matrix Sample Stability Processed Sample Stability

Results and Data

- Method Validation results of the LC-MS/MS method

The LC-MS/MS method validation results met the criteria set forth in the current FDA and EMA BMV guideline.
See representative traces of LGX818 (**Figure 2A**) and MEK162 (**Figure 2B**) at LOQ.

- %Fu - Time Profile of LGX818 and MEK162 in the Equilibrium Dialysis (**Figure 3**)

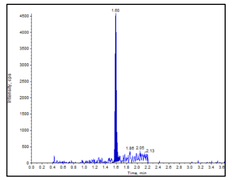

Figure 2A. Trace of LGX818 at LOQ

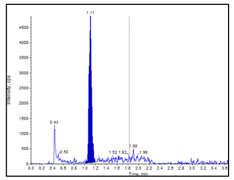

Figure 2B. Trace of MEK162 at LOQ

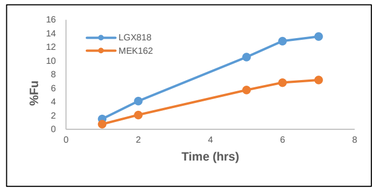

Figure 3. %Fu-Time Profile of the Equilibrium Dialysis

- 7 Hours Incubation stability of LGX818 **(Figure 4A)** and MEK162 **(Figure 4B)** in plasma and PBS (pH=7.4)

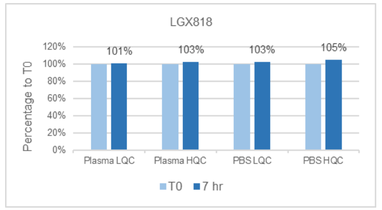

Figure 4A: 7 hrs Incubation Stability of LGX818

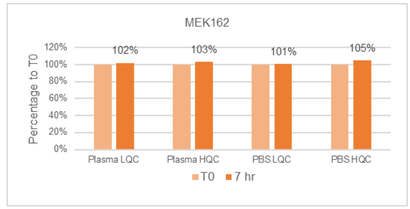

Figure 4B: 7 hrs Incubation Stability of MEK162

- %Fu (**Figure 5A**) and CV of %Fu **(Figure 5B)** of LGX818 and MEK162 (3 runs, 6 replicates at each QC Level)
- %Recovery of the plasma protein binding (PPB) experiment (**Figure 6**) at LQC and HQC levels

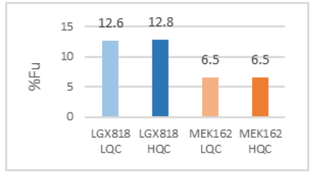

Figure 5A: %Fu at LQC and HQC

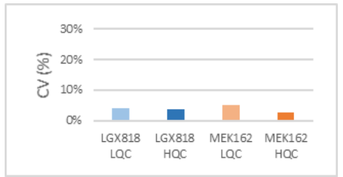

Figure 5B: CV of %Fu at LQC and HQC

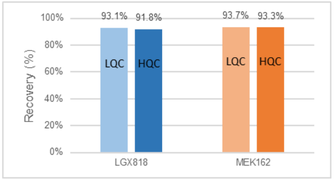

Figure 6: %Recovery of PPB at LQC and HQC

Conclusions

- A sensitive, accurate, precise and specific LC-MS/MS method has been validated for the determination of LGX818 and MEK162 simultaneously in human plasma (K_2EDTA) and PBS (1:1, v/v) mixture;
- A rapid equilibrium dialysis (plasma vs phosphate buffer solution) was developed to evaluate the plasma protein binding of LGX818 and MEK162, with the establishment of dialysis equilibrium time and the evaluation of stability in incubation condition. Low variability in %Fu and good %Recovery were achieved with the developed assay.