Determination of Drugs with Poor Solubility in hERG External Solution by LC-MS/MS to Support hERG Potency Assessment

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Abstract

ICH S7B and ICH E14/S7B Q&As recommend verifying drug concentrations exposed to the recorded cells during hERG block potency assessment. One of the challenges of concentration verification is quantifying drugs that are poorly soluble in the study solutions. Lopinavir and Ritonavir (LOP, RIT) are poorly soluble in water, their solubilities in hERG solution are unknown but expected to be limited. Sensitive LC-MS/MS methods for LOP and RIT have been developed and validated over a concentration range of 10-500 nM. A special sample handling was applied to collect samples that reflect only free molecules . Analytes were stable for 3 freeze/thaw cycles, 6 hours at RT, 99 days at -80 °C. The developed methods were used to measure drug concentrations exposed to the recorded cells during real patch clamp experiments to calculate drug potencies; IC_{50} was found to be 5.1 µM and 14.3 µM, for LOP and RIT, respectively. The established protocol can be generalized to drugs with limited solubility to ensure that concentrations measured from drug solution samples reflect solubilized molecules that can interact with hERG channels.

Introduction

ICH S7B recommends assessing hERG channel block using patch clamp method to understand the risk of clinical QT_C prolongation and the rare but potentially fatal ventricular tachyarrhythmia, Torsade de Pointes. Because drug concentrations exposed to the recorded cells can differ from nominal due to non-specific binding, instability, and experimenter-induced errors, ICH S7B and ICH E14/S7B Q&As recommend verifying drug concentrations exposed to the recorded cells to improve the accuracy of the hERG block potency assessment. Quantifying concentrations of drugs that are poorly soluble in the solutions used to assess hERG block is challenging because samples may contain solutes and insoluble drug particles that are solubilized during sample processing resulting in inaccurate measurements of cell exposure. Lopinavir and ritonavir (LOP, RIT) are poorly soluble in water. Their solubilities in hERG solution are unknown but expected to be limited. The aims of this study are to develop and validate LC-MS/MS methods for two drugs with limited solubilities, to quantify and verify drug concentrations exposed to the recorded cells during the hERG block potency assessment; and to develop an approach to collect and analyze samples that reflect only free molecules to ensure accuracy of the in-vitro real patch clamp experiment data.

Materials and Methods

Instrumentation: Sciex 6500+ Triple-quad with UPLC system, using positive Electrospray MRM mode.

Chromatography:

- Phenomenex Kinetex XB-C18; 2.1x50 mm; 1.7 µm column, at RT
- Mobile phases: 10 mM Ammonium Format with 0.1% FA in water (A), and 0.1% FA in acetonitrile (B)

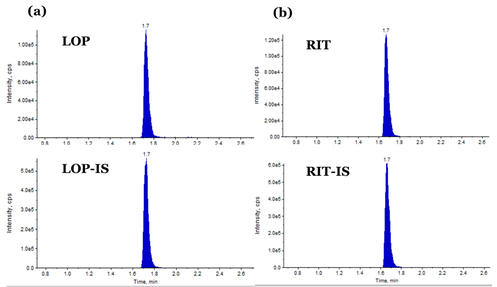

Figure 1. LC-MS/MS chromatogram of 10 nM analyte and corresponding internal standard (a) LOP, (b) RIT

Sample Collection:

- Drug solutions were prepared by prolonged stirring for 1.0 hour
- Filtration through 0.2 µM filters was applied to remove precipitates.
- Absence of precipitates was verified using light scattering method.
- Samples were collected in low-binding tubes and stored at -80 °C
- Real samples (during patch clamp recording) and satellite samples (with no recording) were collected.

Dilution with organic solvents:

- Samples were diluted with different organic solvents (methanol, acetonitrile and a mixture of methanol/acetonitrile to select the best solvent for sample dilution.
- Percentage of organic solvent in the final extract was also evaluated.

Sample Processing:

- Calibration curves were prepared at relatively lower concentrations (10-500 nM) to ensure maximum solubility.
- Dilution linearity was evaluated for up to 20 µM.
- Calibrators (CCs), quality control samples (QCs) and study samples were treated (1:2) with 50/50 acetonitrile/methanol, v/v, and then further diluted with internal standard solutions before LC-MS/MS analysis.

Results and Discussion

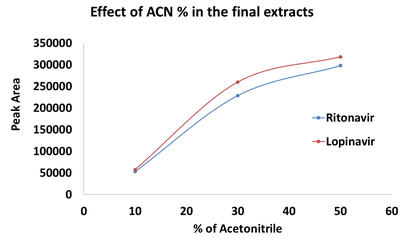

Figure 2. Effect of organic solvent dilution.

Higher percentages of organic solvent in the final extracts showed higher response due to limited solubility of the tested drugs in hERG solution

Method Validation

Table 1. LOP and RIT Validation Summary

Validation Item	Summary
Linearity, Precision and Accuracy	4 acceptable runs
Freeze/Thaw	3 cycles (-80 ◦C /RT) in LoBind tubes
Bench Top stability	6 hours at RT in LoBind tubes without treatment
Dilution Linearity	50-fold dilution
Autosampler Stability	24 hours at 2-8 ◦C
Long Term stability	99 days at -80 ◦C in LoBind tubes
Note: Samples were treated (1:2) with 50/50 acetonitrile/methanol, v/v in the original LoBind tube	

Table 2. Precision (%CV) and % Accuracy of Stability Experiments

		Lopinavir		Ritonavir	
Stability test	Spiked QC concentration (nM)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
Auto-sampler	LQC (30)	4.49	106.38	4.25	106.01
	HQC (400)	4.72	105.51	3.98	103.66
Freeze-thaw	LQC (30)	4.94	104.63	4.23	107.72
	HQC (400)	7.23	98.81	5.23	104.31
Bench-top	LQC (30)	4.62	93.22	7.57	101.22
	HQC (400)	5.88	102.64	6.29	105.45
Long-term	LQC (30)	4.51	104.60	7.64	109.00
	HQC (400)	3.75	97.14	3.00	96.54

Patch Clamp Experiment

The concentrations of LOP and RIT determined by LC-MS/MS from real experiment samples were used to plot the concentration-inhibition curves and to calculate **IC_{50}** (half inhibitory concentration, reported with ±95% confidence interval)

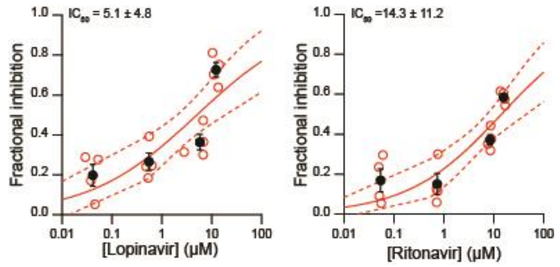

Figure 3. Concentration-inhibition plots for LOP (left) and RIT (right) in hERG external solution. Open red symbols represent individual data points, solid symbols represent (Mean ± Standard Error), The fit to the Hill equation is shown with solid red curve; the upper and lower 95% CI bands of the fit are shown with the dotted red curves.

Conclusion

- LC-MS/MS methods were developed and validated for Lopinavir and Ritonavir analysis in hERG external solution.
- A Bioanalytical-Electrophysiology approach has been established for sample handling and collection to ensure absence of insoluble drug particles during the experiment.
- The validated methods were applied to verify Lopinavir and Ritonavir concentrations to which cells are exposed to improve accuracy of hERG channel block assessment.
- The established approach can be generalized to drugs with solubility issues, to conduct hERG assays to ensure that measured concentrations reflect solubilized molecules that can interact with hERG channels.

References:

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