Bioanalytical assay for the measurement of lopinavir-ritonavir in plasma to support the effective treatment of COVID-19

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Introduction: Lopinavir-ritonavir (Kaletra™) has been proposed as a treatment for COVID-19 on the basis of in vitro activity, preclinical studies, and observational studies. To define optimised dosing regimens a bioanalytical assay to measure therapeutic concentrations of lopinavir and ritonavir in plasma was developed and validated.

Methodology:

Instrument Parameters & Chromatography Settings: Shimadzu Nexera liquid chromatograph (LC) equipped with a Shimadzu 8030+ tandem mass spectrometer (MSMS) detector. Lopinavir and ritonavir were monitored in positive mode electrospray using selective reaction monitoring (SRM) of 629.2→155.2 and 721.25→296.2, respectively. The internal standard [2H8]-lopinavir and [13C3]-ritonavir were monitored using SRM in positive mode at 637.3→163.3 and 724.35→268.2, respectively. LC conditions: The mobile phase was a gradient of acetonitrile containing 0.1% formic acid in water (v/v) with a run time of 5.5 minutes; Analytical column: Xbridge BEH C18, 2.1 x 30 mm (2.5 μm), Flow rate: 0.4 mL/min; Sample preparation: Plasma (10 μL) was spiked with the internal standards ([2H8]-lopinavir and [13C3]-ritonavir and the proteins were precipitated using acetonitrile.)

Results: Bioanalytical validation testing across the range of 0.5 to 50 µg/mL for lopinavir and 0.05 to 5 µg/mL for ritonavir met acceptance criteria for precision (within 3.4%) and accuracy (range 101 to 108%). The method was suitable for a range of patient plasma, with selectivity < 12% at 0.5 µg/mL for lopinavir and at 0.05 µg/mL for ritonavir. Stability was met at all conditions tested (bench top for 4 h, three freeze-thaw cycles and autosampler stability for 4 days). The lopinavir and ritonavir was found to be stable in liquid whole blood samples for 24 h at 5 °C.

Conclusion: A bioanalytical method has been established and validated to demonstrate performance to industry standards (US FDA 2018). This assay will be used in a RBWH Foundation funded project which aims to understand the impact of life-support on the dosing of lopinavir-ritonavir for critically ill patients.

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