Poster

[P25-3] P25-3: Anti-infective drugs (3): TB drugs

Chair: Masahiro Kobayashi, Japan

Mon. Sep 25, 2017 12:30 PM - 1:30 PM Annex Hall (1F)

(Mon. Sep 25, 2017 12:30 PM - 1:30 PM Annex Hall)

[P25-3-5] Development and validation of anti-TB drugs in plasma and tissue to support pharmacokinetic-driven studies

Mark A. Marzinke[1], Pamela Hummert[2] (1.Johns Hopkins University, 2.Johns Hopkins University)

Keywords: Tubcerulosis, Mass Spectrometry, Validation, LC-MS/MS

Background

Tuberculosis, an infection caused by Mycobacterium tuberculosis, is associated with significant disease burden. Current treatment modalities include combinatorial therapies that are associated with significant drug-related toxicities. In an effort to better characterize traditional TB regimens, as well as assess experimental anti-TB drugs, analytical methods are required for drug quantification. This work describes the multiplexed quantification of the common anti-TB medications rifampin, pyrazinamide, ethambutol, as well as the fourth-generation antibacterial agent moxifloxacin and the experimental drug PA-824, in plasma and tissue, via liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods

The anti-TB drugs ethambutol, isoniazid, moxifloxacin, PA-824, pyrazinamide, and rifampin were isolated from blank human K2EDTA plasma or rabbit tissue lung homogenate (0.025 mL) via protein precipitation. Isotopically labeled analogs were used for ethambutol, isoniazid, moxifloxacin, and pyrazinamide; the structural analogs carnidazole &rifamixin-d6 were used for PA-824 and rifampin quantification, respectively. Analytes were chromatographically separated using a C18 50 x 2.1 mm 2.6 m column (Phenomenex Technologies) over a 5 min gradient using a mobile phase system comprised of water and methanol fortified with 0.3% formic acid. Analytes were detected using a SCIEX 5500 QTRAP via electrospray ionization in positive and selective reaction monitoring modes. Assays were validated in accordance with FDA, Guidance for Industry: Bioanalytical Method Validation recommendations.

Results

A multiplexed anti-TB panel was developed and validated for drug quantification in plasma and tissue homogenates. The analytical measuring ranges are as follows: ethambutol: 200 - 10000 ng/mL; isoniazid: 250 - 20000 ng/mL; moxifloxacin: 100 - 10000 ng/mL; PA-824: 250 - 25000 ng/mL; pyrazinamide: 2000 –100000 ng/mL; rifampin: 250 - 50000 ng/mL; the analytical measuring ranges in tissue homogenates were 1/10 those used for plasma. Overall inter-day precision and accuracy for the three QC levels in plasma and tissue for the six analytes was ±11.5% and ±12.3%. Post-column infusion analysis showed minimal relative matrix effects for analyzed anti-TB drugs; however ion suppression was observed for several of the analyzed compounds, with substantial ion suppression of both analyte and internal standard for isoniazid and moxifloxacin in plasma. All stability challenges met acceptability criteria.

Conclusions

The described work illustrates the development and validation of a multiplexed method for the quantification of several current and experimental anti-TB drugs used in the management of a Mycobacterium tuberculosis infection.