Seventeen drugs, one sample: analyzing multiple anti-tuberculosis drugs simultaneously using one method.

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Background: In 2021, a total of 1.6 million people died from tuberculosis (TB), although it is a preventable and curable disease. Depending on susceptibility, TB is treated with a combination of several of 20 anti-TB drugs from the WHO treatment guidelines. Interindividual variability may result in toxicity or ineffective treatment. Therapeutic drug monitoring can be used to optimize dosing and treatment. However, several analyses may be needed, which is time consuming, expensive, and may result in needing multiple samples from a patient. Therefore, we aimed to develop a simple method to analyze all anti-TB drugs in one analysis.

Methods: We developed an LC-MS/MS method in plasma, serum or saliva, allowing simultaneous analysis of 17 anti-TB drugs and 6 metabolites.

Results: 100 µL plasma, serum or saliva was mixed with 450 µL of precipitation solution consisting of stable isotope labelled standards and 50 µL of ion pair solution. The mixture was centrifuged and injected in an LC-MS/MS system. The runtime of any combination of these 17 drugs only takes 1.7 minutes. We validated all standard parameters assuring the quality of our analysis and checked the stability of the samples at different temperatures, allowing extrapolation to resource-limited regions.
With this method, we are able to analyze all first-line and most second-line anti-TB drugs (e.g. bedaquiline, clofazimine, delamanid, ethionamide, gatifloxacin, levofloxacin, linezolid, moxifloxacin, pretomanid, prothionamide, rifabutin, rifapentine, and tedizolid) using a method that was validated according to the EMA Guidance. The four drugs delamanid, ethionamide, protionamide and pretomanid require immediate processing due to limited stability.

Conclusion: We developed a method to analyze 17 anti-TB drugs simultaneously in one sample of plasma, serum or saliva: all first-line drugs, BpaLM (bedaquiline, pretomanid, linezolid, and moxifloxacin) and 9-month all oral regimen for MDR/ RR (multidrug-resistance or rifampicin resistance) TB, and 63% of the longer MDR-TB regimen drugs. This method will save time and will further optimize therapeutic drug monitoring.