LC-MS² based method development for therapeutic drug monitoring

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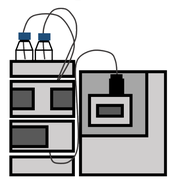
Introduction & Motivation

- Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (*Mtb*) → mostly affects the lungs but can also cause systemic infections
- In 2019, 10 million new cases of tuberculosis were reported worldwide [1]
- Massive increase of multidrug resistant TB worldwide → therapy regimens become more complicated and take at least 20 months according to WHO guidelines
- Lack of adherence or incorrect dosages can lead to development of further resistances during treatment ↔ high doses can lead to increased side effects
- Measuring the pharmacokinetics (PK) and pharmacodynamics (PD) for several drugs over several timepoints as single-analyte assay is resource and time consuming

There is an urgent need to assist clinicians in monitoring all commonly applied combination regimens. Therefore, we developed an LC-MS²-based multi-analyte assay to quantify all anti-TB drugs in one HPLC run.

- Therapeutic drug monitoring (TDM)
- Personalized antibiotic therapy

Human



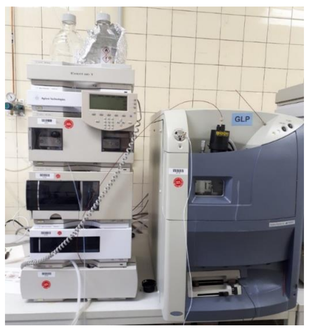
Animal Model

- Test of new antibiotic formulations
- Develop of new antibiotics
- Evaluate novel drug combinations

Method development

Workflow for antibiotic quantification using LC-MS²

- Measurements were done on an Agilent 1100 Series HPLC system using a Merck Milipore SeQuant ZIC-HILIC column
- The HPLC was coupled to a Waters Micromass Quattro Premier XE mass spectrometer via Electrospray



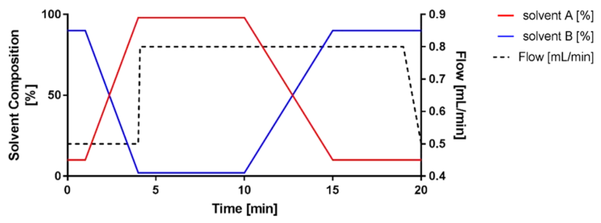


Figure 1: Gradient for the HPLC. Solvent A: 1% FA, Solvent B: ACN

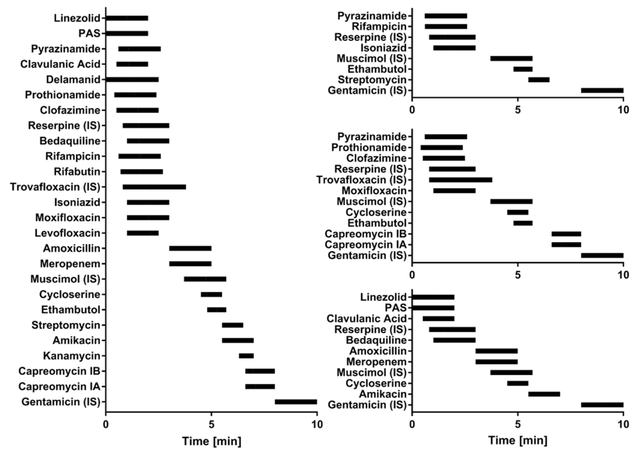


Figure 2: MS channels and detection windows for 26 analytes including 4 internal standards. The channels are combined according to the respective patient's or animal's regimen.

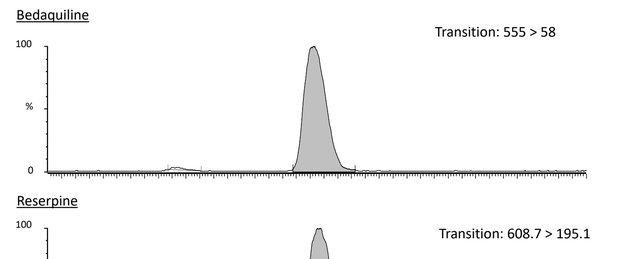


Figure 3: A representative LC-MS² chromatogram of bedaquiline and of the internal standard reserpine.

Animal Model

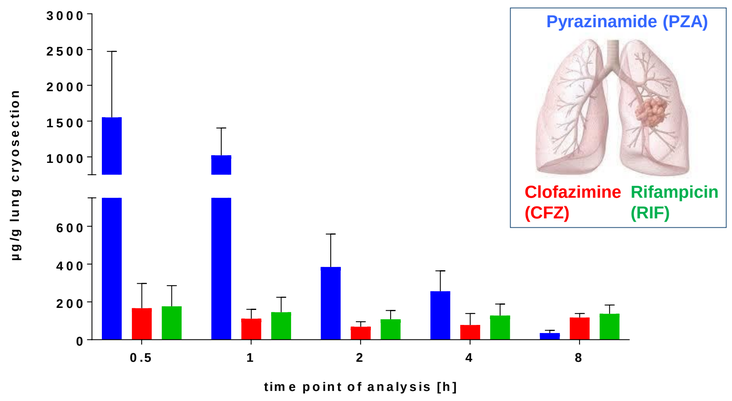


Figure 4: Comparison of the antibiotic concentration at different time points after the last drug administration in lung homogenates of uninfected mice.

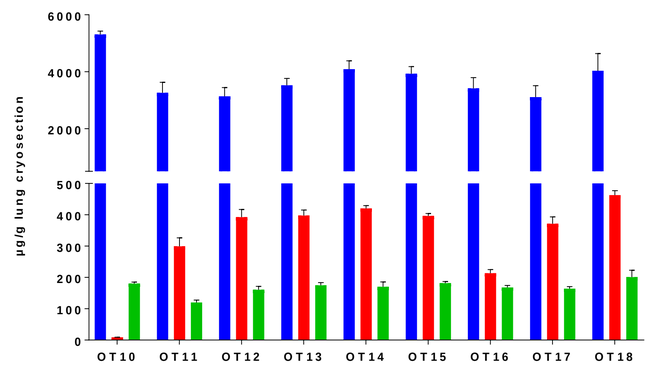


Figure 5: Comparison of the antibiotic concentration one hour after the last drug administration in lung cryosection of uninfected mice. Each lung cryosection was measured as triplicate.

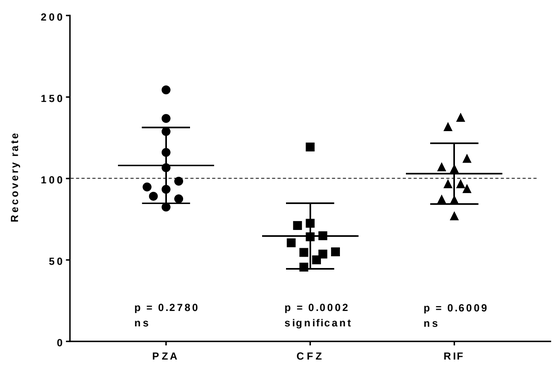


Figure 6: Comparison of the antibiotic concentration of irradiated and non-irradiated lung cryosections of uninfected mice. Each lung cryosection was measured as triplicate.

- We observed a reduction of the PZA concentration over the time whereas the concentration of CFZ and RIF remained stable (*Figure 4*)
- We were able to downscale the extraction procedure to quantify antibiotics in lung cryosections (*Figure 5*)
- Furthermore, we evaluated the influence of gamma irradiation on the drug concentration (*Figure 6*)
  - → gamma irradiation reduced the concentration of clofazimine
  - → In contrast, pyrazinamide and rifampicin were not affected by gamma irradiation

TDM

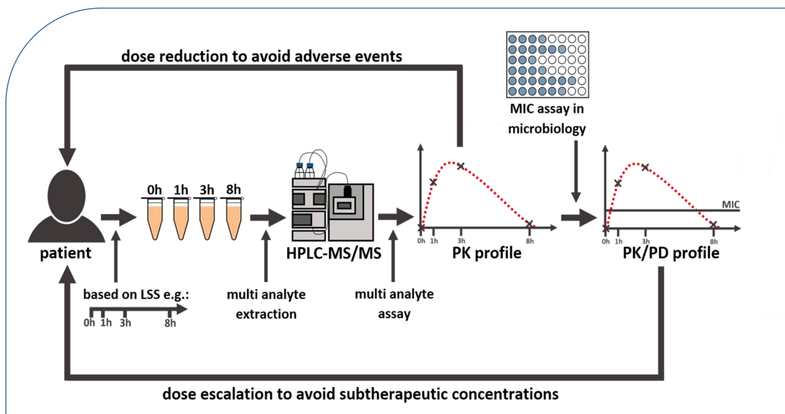


Figure 7: Schematic procedure of therapeutic drug monitoring. [2]

patient

♂, 34 y, XDR, therapy regimen:

- Meropenem
- Moxifloxacin
- Bedaquiline
- Clofazimine
- ....

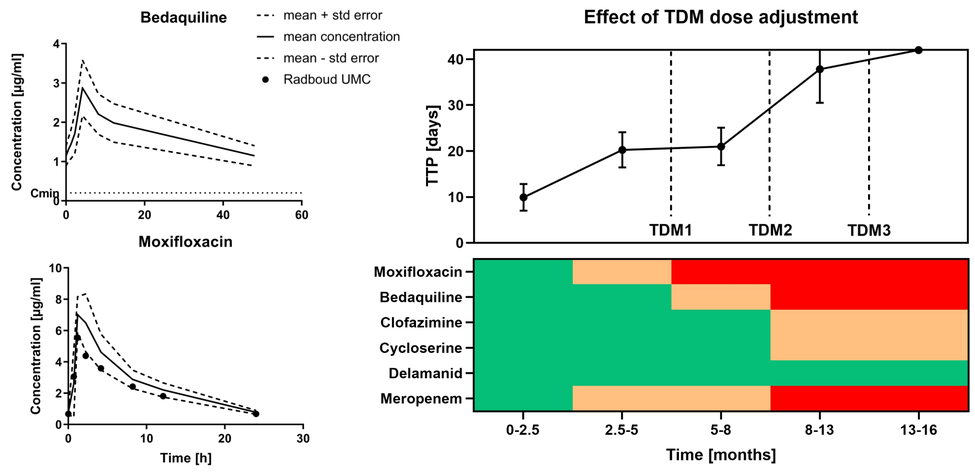


Figure 8: Representative bedaquiline- and moxifloxacin PK profiles at TDM1 and effect of TDM-guided therapy adjustment on the time to culture positivity (TTP) as a surrogate for therapy success. Drug concentrations in plasma were determined with the developed LC-MS² based method. UMC: quality control at the pharmacology lab at Radboud UMC.

- LC-MS² method was applied to determine PK profiles in specific clinical cases (*Figure 8*).

Results

- **The presented LC-MS² method allows to determine 21 different anti-Tb antibiotics plus 4 internal standards**
- **Antibiotics can be measured in different biological matrices – plasma, lung homogenate, cryosections**
- **Irradiation of lung cryosections reduced CFZ concentration but had no influence on the PZA and RIF concentration**
- **LC-MS² method enables PK/PD analysis of TB patients, up to 10 antibiotics plus internal standards in parallel**
- **Method is not restricted to established antibiotics: it can be expanded to new molecules**

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References

[1] Global Tuberculosis Report 2019, WHO
[2] Salzer et *al.*, Respiration 2016

Acknowledgments

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