

# LC-MS/MS method to quantify antibiotic uptake in *E. coli*

**M. García-Rivera<sup>1</sup>, V. Fetz<sup>1,2</sup> and M. Brönstrup<sup>1</sup>**

<sup>1</sup>Helmholtz Centre for Infection Research, Inhoffenstraße 7, 38124 Braunschweig, Germany

<sup>2</sup>Jacobs University Bremen, Campus Ring 1, 28759 Bremen, Germany

E-mail: mariel.garcia@helmholtz-hzi.de

Multidrug-resistant Gram-negative bacteria constitute a major health care issue. Low permeability of the outer membrane of Gram-negative bacteria can challenge antibiotic uptake. In order to support the generation of effective, novel antibiotics, an in-depth understanding of transport processes across the cell wall is needed [1]. With a label-free uptake determination, compounds are not modified in terms of size or charge, and natural uptake mechanisms are not influenced [2-3]. In this respect, a mass spectrometry (MS)-based procedure was developed [4]. This allows not only a direct compound quantification, but also a high sample throughput by measuring several compounds.

Two *E.coli* strains and their omp<sup>1</sup> expressing mutants were incubated with six  $\beta$ -lactam antibiotics and one fluoroquinolone, according to [5]. For a reliable, precise and direct antibiotic uptake determination, LC-MS/MS<sup>2</sup> quantitation methods were developed for every compound. The capability of bacteria to accumulate different compounds was then calculated not only by the effective concentration within the cell (intracellular concentration), but also by how much of the compound was taken up in comparison with the initial available amount (uptake fraction). These results will eventually be useful to determine the effect of antibiotic uptake on specific metabolic responses triggered within Gram-negative bacteria.

## References

- [1] J. M. Pagès, C. E. James, and M. Winterhalter, *Nature Reviews Microbiology* **6**, 895-903 (2008).
- [2] H. Cai, et al., *Anal Biochem* **2**, 321-325. (2009)
- [3] T. D. Davis, C.J. Gerry, and D.S. Tan, *ACS Chem Biol* **9**(11), 2535-2544 (2014).
- [4] A. Heumann, Master thesis, TU Braunschweig (2015)
- [5] S. Kaščáková, L. Maigre, J. Chevalier, M. Réfrégiers, J.M. Pagès, *PLoS ONE* **7**(6), e38624 (2012)

---

<sup>1</sup> Outer membrane porin

<sup>2</sup> liquid chromatography coupled with tandem-MS