

Applications - Biomedical and clinical separations/Pharmaceutical analysis

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Title: VALIDATED HPLC METHODS WITH FLUORESCENCE DETECTION FOR THE QUANTITATION OF CIPROFLOXACIN AND AMPICILLIN ANTIBIOTICS IN MOUSE FAECAL SAMPLES TO STUDY BACTERIAL MULTIDRUG RESISTANCY

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Abstract body

Antibiotic resistance is one of the most serious public health challenges in our time, resulting in more and more deaths each year. Multidrug resistant (MDR) bacteria exhibit resistance to multiple classes of antibiotics, which makes it extremely difficult to fight against them. A hot topic in current microbiology is to study the mechanism how multiresistancy evolves and spreads to other bacteria. A possible methodology is to colonize the gastrointestinal tract of laboratory mice with MDR bacteria and after treatment with different antibiotics measure the change in the quantity of colonizing pathogens and their resistance gene copy numbers, as well as the antibiotic concentrations in the stool samples. In medical research the agar diffusion method is a widely accepted technique for faecal antibiotic concentration measurements, but it suffers from low sensitivity and poor reproducibility. HPLC could provide the desired sensitivity, however the overwhelming majority of bioanalytical methods in the literature is measuring antibiotic concentrations in serum/plasma or urine. Very few methods are described for faecal samples and even less are validated. In the light of this, we have developed and validated two separate HPLC methods with

fluorescence detection that can reliably and sensitively quantitate a fluoroquinolone antibiotics ciprofloxacin and a penicillin derivative, ampicillin in limited amounts of mouse faecal samples.

For the determination of ampicillin mouse faecal pellets were extracted with acetonitrile: water mixture after homogenization and derivatized with formaldehyde. The fluorescent derivative of ampicillin was separated on a Kinetex EVO C18 column and detected at $\lambda_{\text{ex}}=346$ nm and $\lambda_{\text{em}}=422$ nm. Ciprofloxacin was extracted from mouse faeces with 0.1 M phosphoric acid using an ultrasonic disintegrator. The sample extract was separated on the same column as ampicillin and detected at 310 nm excitation and 445 nm emission wavelengths.

The two bioanalytical methods were validated. Using only 10 mg of mouse stool the methods exhibited high sensitivity; the LLOQ was 0.1 ppm for both ampicillin and ciprofloxacin. Linearity of the methods was proven between 0.1-1 ppm for ciprofloxacin and between 0.1-2 ppm for ampicillin using matrix-matched calibration samples. Within-day repeatability and between-day reproducibility of the methods were below 6 and 13% for ampicillin and 5 and 10% for ciprofloxacin, respectively.

The validated methods have been successfully applied in a microbiological research which was focusing on the effects of per os antibiotic treatment in mouse model colonized with a MDR *Klebsiella pneumoniae* strain.