

MATERIAL SUPPLEMENTARY

Extended materials and methods

1- Reagents

Amoxicillin (Clamoxyl® 500 mg) and ceftriaxone (Ceftriaxone 1g Mylan®, powder for solution for injection) were respectively purchased from GlaxoSmithKline (Brentford, United Kingdom) and Mylan (Canonsburg, Pennsylvania, USA). Colloidal silica, petrolatum and carmine powder were obtained from Cooper (Melun, France). Sterile water was purchased from BBraun (Melsungen, Germany). Chemicals and reagents used for HPLC-MS/MS were all of analytical grades and included water, methanol and acetonitrile purchased from Carlo Erba (Fontenay-aux-Roses, France). Formic acid, and ammonium formate were purchased from VWR Chemicals (Leuven, Belgium). Amoxicillin-d4 and ceftriaxone-d4 were purchased from Toronto Research Chemicals (Toronto, Canada).

2- Analytical method

2-1- Chromatographic conditions

Amoxicillin and Ceftriaxone were determined by liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) using a Shimadzu prominence HPLC system (Shimadzu®, Kyoto, Japan) coupled to a 4500 QTRAP triple quadrupole mass spectrometer (Sciex®, Framingham, Massachusetts, USA), equipped with an electrospray ionization source operating in the positive ion mode. An Alltima HP C18 HL 3µm 150 × 3 mm column (Alltech®, Grace Discovery Sciences) was maintained at 30°C with a flow rate of 0.6 mL/min for 5.5 minutes using an elution gradient. The mobile phase consisted of 30% methanol with 0.2% formic acid from acetonitrile and 70% 2 mM ammonium formate with 0.2% formic acid in water (70/30

v/v) from 0 to 1.5 min, 100% methanol with 0.2% formic acid from 1.5 to 3.5 min and 30% methanol with 0.2% formic acid from acetonitrile and 70% 2 mM ammonium formate with 0.2% formic acid in water (70/30 v/v) from 3.6 to 5.5 min.

Amoxicillin-d4 and ceftriaxone-d4 were employed as internal standards. Analyst 1.6.2 software (Sciex®, Framingham, Massachusetts, USA) was used for data acquisition and analysis. Amoxicillin and ceftriaxone detection and quantification were performed in the multiple reaction monitoring mode (MRM) using respectively the protonated $[M+H]^+$ amoxicillin and amoxicillin-d4 (m/z 365.9 and 369.9 respectively), and ceftriaxone and ceftriaxone-d4 precursor ions (m/z 554.8 and 558.8). The ion transitions monitored were m/z 365.9 \rightarrow 349.0 and m/z 365.9 \rightarrow 113.8 for quantitation and confirmation of amoxicillin, respectively. The ion transitions monitored for quantification and confirmation of ceftriaxone were respectively m/z 554.8 \rightarrow 395.8 and m/z 554.8 \rightarrow 323.8. Source parameters were optimized as follow: ion spray voltage: 5500V; nebulization gas: 40 psi; desolvation gas: 65 psi; curtain gas: 25 psi; source temperature: 500°C.

2-2- Sample preparation

2-2-1- Calibrators and validation standards

Stock solutions of amoxicillin and ceftriaxone (1 mg/mL, A1) were obtained by solvating 1 mg of each compound in 1 mL of water. Stock solutions were packaged in amber bottles and stored in the freezer (-20°C). 400 μ L of stock solutions (A1) were diluted in volumetric flask to reach 20 μ g/mL (A2). Stock solutions A2 were diluted in 5 mL volumetric flask to obtain calibrators (0, 1, 2, 4, 8, 16 and 20 μ g/mL) and validation standards (3, 10 and 18 ng/mL). A1, A2, calibrators and validation standards were freshly prepared on each day of analysis.

2-2-2 Internal standards

Internal standards were prepared by solvating 1 mg of amoxicillin-d4 and ceftriaxone-d4 with 1 mL HPLC grade methanol (1 mg/mL), followed by the dilution in 200 mL methanol in volumetric flask to reach 5 µg/mL. The internal standard was added to calibrators and validation standard samples.

2-2-3 Samples

Amoxicillin hard capsules were opened, and the contained powder was weighted. The amount of powder corresponding to 1 mg (for 5 mg capsules) or 10 mg (250 mg capsules) of amoxicillin was diluted in 10 mL volumetric flasks with 50:50 water/acetonitrile mixture, followed by a dilution in water to reach a theoretical concentration of 10 µg/mL. Ceftriaxone was diluted in water to reach a theoretical concentration of 10 µg/mL. For ceftriaxone patch tests in petrolatum, a prior dissolution with dichloromethane was performed.

3- Data analysis

Values are reported as mean and standard deviations (\pm SD) in graphs. Statistical analysis and graphs were performed using GraphPad Prism, version 8.3.0 (GraphPad Software, La Jolla, USA).