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Simultaneous determination of ceftaroline, daptomycin, linezolid and rifampicin concentrations in human plasma by on-line solid phase extraction coupled to high-performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* infection is a serious clinical problem worldwide. Ceftaroline, daptomycin, linezolid in combination with rifampicin are particularly used in this indication. To allow monitoring of these antibiotics, an on-line solid phase extraction coupled to high-performance liquid chromatography–tandem mass spectrometry assay requiring a 100 μL aliquot of human plasma has been developed. Besides, significance of 25-*O*-desacetylrifampicin concentrations was evaluated.

Sample pre-treatment is limited to protein precipitation with methanol. After centrifugation 10 μL of supernatant are injected into the chromatographic system, which consists of an on-line solid phase extraction followed by a separation on a phenyl-hexyl column and detected by a tandem mass spectrometer. Plasma drug concentrations were determined by multiple reaction monitoring in positive ion mode, and assay performance was evaluated. 25-*O*-Desacetylrifampicin activity, was compared to rifampicin using a microbiological method.

Sample preparation using methanol precipitation followed by solid-phase extraction yielded good recovery and ionization efficiency, with chromatographic separation achieved within 3 min per sample. Within-run and between-run precisions ranged respectively from 1.22% to 9.35% and from 1.61% to 9.36%. Lower limits of quantification were 0.04 mg/L for linezolid, 0.1 mg/L for rifampicin, 0.2 mg/L for ceftaroline and 0.5 mg/L for daptomycin. It appears that 25-*O*-desacetylrifampicin displays a substantial intrinsic bactericidal activity against *S. aureus*.

This assay provides simple, rapid, sensitive and accurate quantification of the four antibiotic drugs and one metabolite and can be routinely used to monitor drug concentration in methicillin-resistant *S. aureus* infected patients.

1. Introduction

According to the 2013 European Centre for Disease Prevention and Control report, methicillin-resistant *Staphylococcus aureus* (MRSA) range between 0 and 64.5% of the isolated *S. aureus* strains in European countries [1]. Since the beginning of the 2000s in United States of America, the appearance of community-associated MRSA infections represents a major public health issue [2]. Glycopeptide antibiotics and especially vancomycin are the most employed agents in case of MRSA infections. However, given the toxicity of these antibiotics, the growing risk of vancomycin-resistant *S. aureus* and the uncertainties concerning efficacy of these drugs, novel agents were developed in the fifteen past years to struggle against MRSA and other multi-drug resistant bacteria infections [3]. Among them daptomycin (lipopeptide),

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Table 1
Antibiotic drugs and MRM transitions used for detection, DP, EP, CEP, CE and CXP for API 3200, and retention times for the phenyl-hexyl HPLC column.

	MRM Transition (*m*/*z*) Precursor ion—product ion	Retention time (min)	DP (V)	EP (V)	CEP (V)	CE (eV)	CXP (V)
Ceftaroline	605–208.1	0.96	51	9.5	28	39	6
Daptomycin	811.1–159.1	0.96	51	12	30	65	4
Rifampicin	823.3–791.3	0.95	76	3.5	70	27	16
25-*O*-Desacetylrifampicin	781.4–749.3	0.95	36	7.5	30	23	10
Llinezolid	338.1–296.1	0.95	61	10.5	16	23	10
$^{2}H_4$-Ceftaroline	609.0–212.1	0.97	51	9.5	28	39	6
$^{2}H_8$-Rifampicin	831.3–799.3	0.95	76	3.5	70	27	16

DP: declustering potential, EP: entrance potential, CEP: cell entrance potential, CE: collision energy, CXP: cell exit potential.

linezolid (oxazolidinone) and ceftaroline (advanced-generation cephalosporin) are increasingly used. Added to them, rifampicin (rifamycin), usually used to treat tuberculosis infections is also employed. Its main metabolite, 25-*O*-desacetylrifampicin, appears to have an intrinsic antibacterial activity but no published studies prove that [4]. To maximize the follow-up of the infected patients by the way of therapeutic drug monitoring, measurement of plasma concentrations could be an interesting tool. Indeed, there are demonstrated relations between plasma concentrations and efficacy and/or toxicity [5–12]. Many assays using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) were reported to measure simultaneously with other agents or alone these antibacterial drugs but currently none allows the measurement of ceftaroline plasma concentrations [13–33]. Here we proposed an on-line solid phase extraction (SPE) coupled to LC–MS/MS turnkey method with a simple sample pre-treatment which can be easily used for the therapeutic drug monitoring of four anti-MRSA agents concentrations in the plasma of infected patients. This method was validated according to the FDA, EMEA and French committee of accreditation guidelines [34–36]. Besides, we proposed an evaluation of the intrinsic bactericidal activity of the 25-*O*-desacetylrifampicin.

2. Material and methods

2.1. Chemicals and reagents

Methanol, acetic acid and ammonium acetate were purchased from VWR International (Fontenay-sous-bois, France). Methanol and water were HPLC grade. Each molecule is measured using a deuterated internal standard: ceftaroline (purity > 99%) was graciously given by AstraZeneca (Rueil-Malmaison, France), daptomycin (Cubicin® 350 mg), rifampicin (Rifadine® 600 mg) and linezolid (Zyvoxid® 2 mg/mL) were respectively purchased from Novartis (Rueil-Malmaison, France), Sanofi-Aventis (Paris, France), and Pfizer (Paris, France). 25-*O*-Desacetylrifampicin (purity > 99%), ceftaroline-d4 (purity: 97.5%, isotopic purity ≥ 99%) and rifampicin-d8 (purity: 97.5%, isotopic purity ≥ 99%) were purchased from Alsachim, (Strasbourg, France). Features are listed in Table 1.

2.2. Standard solutions and ISs

Stock solutions were prepared as follows: rifampicin (methanol including 500 mg/L of ascorbic acid) and daptomycin (water) at a concentration of 10,000 mg/L, linezolid (methanol) and 25-*O*-desacetylrifampicin (methanol including 500 mg/L of ascorbic acid) at a concentration of 2000 mg/L and ceftaroline (methanol), ceftaroline-d4 (methanol) and rifampicin-d8 (methanol including 500 mg/L of ascorbic acid) at a concentration of 1000 mg/L.

For the calibration samples, a working solution was firstly prepared by diluting the stock solution in methanol to a final concentration of 1000 mg/L for daptomycin, 400 mg/L for ceftaroline, 80 mg/L for linezolid and 200 mg/L for rifampicin and 25-*O*-desacetylrifampicin. 50 μL of this solution was mixed with 450 μL of drug-free human plasma (collected with K_3EDTA as anticoagulant) to obtain the highest calibration sample. The other calibration samples were prepared by successive dilutions in drug-free plasma from the highest calibration sample.

2.3. Calibration curves, quality controls and sample preparation

Eight-point calibration curves (0.2, 0.5, 1, 2, 5, 10, 20, 40 mg/L for ceftaroline, 0.5, 1.25, 2.5, 5, 12.5, 25, 50, 100 mg/L for daptomycin, 0.04, 0.1, 0.2, 0.4, 1, 2, 4, 8 for linezolid and 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 20 mg/L for rifampicin and 25-*O*-desacetylrifampicin) were calculated (area ratio using the internal standard versus nominal concentration) and fitted either by a linear regression or by a quadratic regression. The concentrations were back-calculated and the model with the lowest deviation between the calculated and nominal concentrations was retained.

For the quality controls, 3 different levels solutions (high, medium and low) were prepared by diluting the working solution in drug-free human plasma to respective-final concentrations of 32, 3.2 and 0.25 mg/L for ceftaroline, 80, 8 and 0.625 mg/L for daptomycin and 16, 1.6 and 0.125 mg/L for linezolid, rifampicin and 25-*O*-desacetylrifampicin.

The blood samples with K_3EDTA as anticoagulant were centrifuged at 1800 × *g* for 10 min at 4 °C. 100 μL of plasma was treated with 200 μL of methanol as precipitation reagent including 3.3 mg/L of ceftaroline-d4 and rifampicine-d8, used as internal standards respectively for ceftaroline and linezolid and for daptomycin, rifampicin and 25-*O*-desacetylrifampicin, in a 1.5 mL polypropylene tube (Eppendorf, Le Pecq, France). Samples were immediately vortexed and then centrifuged at 13 000 × *g* for 15 min at 4 °C. Then, 200 μL of supernatant was transferred into a 200 μL sample vial (Interchim, Montluçon, France) and 10 μL was injected into the chromatographic system.

2.4. On-line solid phase extraction coupled to LC–MS/MS conditions

2.4.1. Chromatographic conditions

Chromatographic system consisted of Agilent (Palo Alto, CA, USA) 1200 Series components including binary pump, isocratic pump, column oven, a ten-port switching valve and an autosampler. The hardware configuration included an Applied Biosystems (Foster City, CA, USA) API 3200® equipped with a TurboIonspray source. On-line SPE was performed using a perfusion column (POROS® R1/20, 2.1 mm × 30 mm, Applied Biosystems, Foster City, CA, USA). The HPLC column was a short phenyl-hexyl column (phenomenex luna 5 μm phenyl-hexyl, 2 mm × 50 mm, Aschaffenburg, Germany). Data analysis was performed using the Analyst 1.4.2 software package (Applied Biosystems, Foster City, CA, USA).

The chromatographic conditions and the pumps and valves configurations are shown in Fig. 1. A 10 μL aliquot of supernatant from each prepared sample was injected. The binary pump supplied eluent A (water–acetic acid (99.9:0.1, v/v)-ammonium acetate 10 mM) for drug binding onto the online-SPE column