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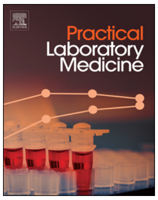

Bioanalytical development and validation of liquid chromatographic–tandem mass spectrometric methods for the quantification of total and free cefazolin in human plasma and cord blood

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ABSTRACT

Objectives: Cefazolin is a commonly prescribed β-lactam antibiotic for prophylaxis against skin infections following surgery, including caesarean sections. Assessment of maternal and neonatal exposure is important for correlating drug concentrations to clinical outcomes. Thus, bioanalytical methods for the quantification of both total and free cefazolin in maternal plasma and cord blood can assist in the comprehensive evaluation of cefazolin exposure.

Design and methods: Specimen preparation for the measurement of total cefazolin was performed via protein precipitation with acetonitrile containing the internal standard cloxacillin. Ultrafiltration was used to isolate free cefazolin. Processed samples were analyzed on a Prelude SPLC system coupled to a TSQ triple quadrupole Vantage mass spectrometer. Methods were validated following FDA bioanalytical guidelines.

Results: The analytical measuring ranges of these methods were 0.48–480 μg/mL and 0.048–48 μg/mL for total and free drug, respectively. Calibration curves were generated using $1/x^2$ weighted linear regression analysis. Total cefazolin demonstrated inter- and intra-assay precision of ≤20% at the LLOQ and ≤11.2% at other levels. Free cefazolin demonstrated inter- and intra-assay precision of ≤18.5% at the LLOQ and ≤12.6% at other levels, respectively. Accuracy (%DEV), carryover, matrix effects, recovery and stability studies were also acceptable based on FDA recommendations. Furthermore, it was demonstrated that samples prepared in cord blood can be accurately quantified from an adult plasma calibration curve, with recoveries ≤9.1% DIF and ≤11.9% DIF for total and free cefazolin, respectively.

Conclusions: The described LC–MS/MS methods allow for the measurement of total and free cefazolin in both plasma and cord blood.

1. Introduction

Cephalosporin antibiotics have become one of the most commonly prescribed classes of β-lactams due to their broad spectrum of anti-bacterial activity, low toxicity profile, and ease of administration [1,2]. Consequently, cephalosporins have been utilized for the treatment of a number of soft tissue and skin infections in both perioperative and post-surgical settings, particularly as prophylactic agents [1,3–5]. Cephalosporins are semi-synthetic compounds initially derived from the fungus *Cephalosporium acremonium* [6]. Structurally, these drugs are comprised of a β-lactam moiety fused to a 3,6-dihydro-2H-1,3-thiazine ring [1]. There are several generations of cephalosporin antibiotics, and family members are stratified based on their anti-bacterial activity and route of administration.

Cefazolin, a first-generation cephalosporin, is widely used to manage skin infections, and has also shown therapeutic efficacy in the treatment of pulmonary infections and methicillin-susceptible *Staphylococcus aureus* [7,8]. Like other first-generation cephalosporins, cefazolin is effective in treating Gram-positive bacteria, but does not elicit equally effective bactericidal effects against Gram-negative bacteria [6,9]. Moreover, cefazolin has been administered prophylactically to prevent post-surgical skin infections, including those

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incurred during cesarean deliveries [10,11]. Cesarean delivery is the primary risk factor for postpartum maternal infections caused by Gram-positive bacteria, and studies have demonstrated that the infection rate in a post-cesarean setting can be as high as 38% [12]. Notably, the prophylactic administration of cefazolin prior to skin incision reduced post-cesarean morbidity as well as endometritis, and has been recommended by the American College of Obstetrics and Gynecology as a perioperative prophylactic agent during cesarean deliveries [11–13]. While the majority of the drug is predominantly protein bound by albumin (~90% bound), cefazolin elicits its antimicrobial effects in the unbound, or free, form [14–16]. Thus, determination of both total and free cefazolin concentrations may be helpful in better characterizing the pharmacokinetics of the cephalosporin in a variety of clinical scenarios, including in a post-cesarean setting. Cefazolin quantification in the mother, as well as the neonate, can provide clarity on neonatal drug exposure, as well as its potential impact on post-delivery outcomes, including the prevention of neonatal sepsis [17]. Neonatal drug exposure measurement has been assessed in many matrices including urine, blood, meconium, hair, and umbilical cord blood. Umbilical cord blood is a convenient matrix to assess neonatal exposure because it should reflect recent changes in neonatal exposure at the time of delivery without having to perform a venipuncture.

Cefazolin quantification has been previously reported using high performance liquid chromatographic (HPLC) [18–20] or liquid chromatographic–tandem mass spectrometric (LC–MS/MS) [21–25] approaches. However, none of the aforementioned methods have extensively evaluated the acceptability of cord blood as a matrix for cefazolin determination. While several methods provide strategies for directly quantifying free drug concentration, only the methods published by Zhang et al. [21] and Douglas et al. [23] utilized tandem mass spectrometry as an analytical detector [18–20,22]. Here we present a comprehensive method describing the development and validation of LC–MS/MS methods for the quantification of total and free cefazolin via ultrafiltration in plasma and cord blood. The method uses a low volume of specimen for determination, requiring less than 250 μL for determination of both total cefazolin and free drug within a sample.

2. Materials and methods

2.1. Chemicals

Cefazolin (97% purity) as its sodium salt, its structural analogue cloxacillin (94% purity) as its hydrated sodium salt (Fig. 1), and ethylenediaminetetraacetic acid (EDTA) were acquired in powder form from Sigma Aldrich (St. Louis, MO). Drug-free human K_2EDTA plasma was purchased from Biological Specialty Corporation (Colmar, PA). Drug-free human K_2EDTA cord blood plasma was purchased from Golden West Biologicals (Temecula, CA). HPLC-grade water, acetonitrile, and formic acid were acquired from Fisher Scientific (Pittsburgh, PA).

2.2. Preparation of reagents and standards

Concentrations of cefazolin and cloxacillin are presented as their free base forms. Cefazolin working solutions for preparation of calibration standards were prepared for determination of total cefazolin by initially preparing a solution containing 23,800 μg/mL cefazolin in 1:1 acetonitrile:water by weighing standard material and diluting volumetrically. Working solutions were prepared at final concentrations of 48 μg/mL, 480 μg/mL, and 4,800 μg/mL via serial dilution of the initial stock solution. Calibration standards were prepared at final concentrations of 0.48, 2.4, 4.8, 12, 24, 48, 120, 240, and 480 μg/mL by spiking K_2EDTA plasma with the appropriate stock solution. The concentrations reflect the quantity of the cefazolin sodium salt in solution. Total organic volume added to plasma was $<2\%$. Quality control (QC) materials were prepared using working solutions prepared from an independent weighing from the solutions used to prepare calibration standards. Working solutions for preparation of QC materials were prepared at concentrations of 190, 2400, and 48,000 μg/mL in 1:1 acetonitrile:water. Working solutions for the preparation of QC materials were diluted into K_2EDTA plasma at final concentrations of 0.48 (lower limit of quantification, LLOQ), 1.4 (low QC), 18.0 (mid QC), and 410 (high QC) μg/mL.

Cefazolin working solutions for preparation of calibration standards were prepared for the determination of free cefazolin by initially preparing a solution of 1900 μg/mL cefazolin in 1:1 acetonitrile:water by weighing standard material and diluting volumetrically. Working

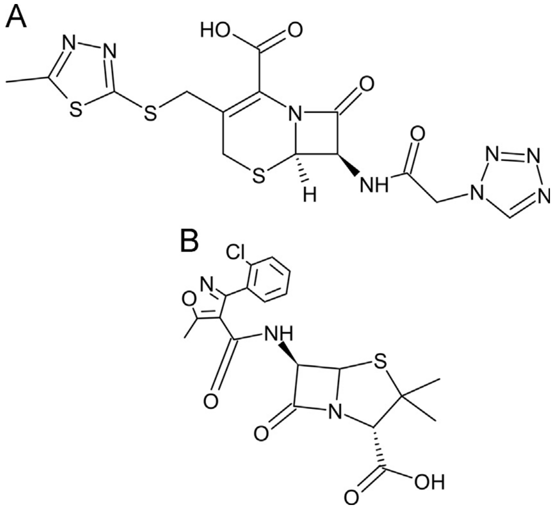

Fig. 1. Structures of (A) the beta-lactam antibiotic cefazolin and its (B) structural analog, cloxacillin.