Journal of Chromatography B, 878 (2010) 2831–2838

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

LC–MS/MS method development and validation for the determination of polymyxins and vancomycin in rat plasma

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ARTICLE INFO

Article history:
Received 14 July 2010
Accepted 23 August 2010
Available online 16 September 2010

Keywords:
LC–MS/MS
Trichloroacetic acid
Polymyxin
Colistin
Vancomycin
Sample recovery
Antibacterial
Peptide drugs

ABSTRACT

Simple, sensitive and robust liquid chromatography–tandem mass spectrometer (LC–MS/MS) methods were developed and validated for the determination of lipopeptide polymyxins and glycopeptide vancomycin in rat plasma. The effect of trichloroacetic acid (TCA) concentration on sample recoveries (peak area of sample recovered from plasma/peak area of sample from neat solvent solutions) was studied and an optimized concentration of 30% TCA were determined that gives the best sample recovery for the peptides from rat plasma. The effect of the TCA concentration on the chromatographic behavior of peptides was studied on a Phenomenex Jupiter C18 5 μ 300 Å 50 mm × 2 mm column using a mobile phase with a pH of 2.8. Other than protein precipitation, TCA also acted as ion pairing reagent and was only present in the samples but not in the mobile phases. The data demonstrated that by increasing the TCA concentration, the analyte retention and sensitivity were improved. The absence of TCA in mobile phase helped to reduce the ion source contamination and to achieve good reproducibility. The plasma method was linearly calibrated from 5 to 5000 ng/mL for polymyxins with precisions to be of 2.3–10.8%, and accuracies to be 91.7–107.4% for polymyxin B1, B2, E1, E2, respectively. For vancomycin the calibration is from 1 to 5000 ng/mL with precisions to be of 7.8–10.3 and accuracies to be 96.2–102.0%. The LLOQs corresponding with a coefficient of variation less than 20% were 7.5, 18.1, 7.3, 5.0 and 1.0 ng/mL for polymyxin B1, B2, E1, E2 and vancomycin, respectively.

1. Introduction

In the past 30 years, the emergence of multi-drug resistance (MDR) bacteria has created a situation in which there are few or no treatment options for infections by certain microorganisms. For example, the emerging MDR Gram-negative bacteria, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, are resistant to all β-lactams, fluoroquinolones, and aminoglycosides [1,2]. Additionally, methicillin resistant *Staphylococcus aureus* (MRSA) has evolved into a significant pathogen among hospitalized patients around the world [3]. Lipopeptide PMXs and glycopeptide VCM interact noncovalently to their target ligands, usually cell-wall or cell-membrane structures. As the noncovalent interactions are nonspecific than covalent interactions, it is more difficult for bacteria to develop resistance to these agents [4]. This mechanist opportunity is used in developing antibacterial peptide drugs against MDR bacteria [5]. This has led to the resurgence in the use of PMX antibiotics which are active against a wide spectrum of Gram-negative bacteria despite their known nephrotoxicity [6,7]. Moreover, there exist renewed interests in the exploration of VCM and other glycopeptides modifications that are active against Gram-positive bacteria [8].

The two clinically used PMXs, PMB and PME, are cyclic lipodecapeptides. In these peptides, the amino acid units 1–3 are linear and 4–10 form a 23-membered ring. Each molecule carries 5 free amino groups and, accordingly, 5 positive charges are present under physiological conditions [9]. The main difference between PMB and PME is in the amino acid components. PMB is comprised mainly of PMB1 and PMB2 [10], and PME (also known as colistin), is comprised mainly of PME1 (colistin A) and PME2 (colistin B) [11,12]. The cationic molecules of PMX compete and displace Ca^{2+} and Mg^{2+} ions, and the hydrophobic segments of PMX microscopically form complexes with bacterial lipopolysaccharide, which causes local disturbance of the cell membrane, and increases cell permeability, cell lysis and death [13–16]. They display sub-micromolar minimum inhibitory concentration (MIC) values against a variety of Gram-negative bacteria [2,6,8,9,17,18].

Currently there is a lack of reliable information concerning the pharmacokinetic data for PMXs in humans [2,10]. PMXs are

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doi:10.1016/j.jchromb.2010.08.037

highly soluble in water and poorly soluble in organic solvents [19]. The unique molecular properties of PMXs present chromatographic challenges with a variety of conventional reverse phase LC columns. Since all the main components of the PMXs possess five free amino groups which tend to adsorb onto silica surface [20], severe peak tailing are observed for untreated PMX samples with LC. Therefore, either derivatization [21] or further purification are required for optimal bioanalysis. Bioanalytical methods such as capillary zone electrophoresis (CZE) [20,22,23], high-performance liquid chromatography (HPLC) with fluorescence detector, UV spectrophotometric detector or scanning fluorescence detector [21,24,25], and LC–MS/MS [26,27] have been used for quantitative analysis. Since CZE and LC with UV and fluorescence detection lack of structure-specific selection [24], and fluorescence detection requires compound derivatization for a sensitive and specific method [21,24,25,28], LC–MS/MS is the choice for pharmaceutical industry because of its high sensitivity and structural specificity. The reappraisal of PMXs as the only available active antibiotics for some bacteria species as well as the combined-drug synergy study of PMXs with other antibacterial compounds [2] demand a simple and accurate analytical method with adequate dynamic range and sensitivity for the determination of PMXs in biological samples.

Recently, LC–MS/MS methods have been developed for quantification of PME (colistin) in milk and animal tissues [27,29]. The methods required the use of strong and highly concentrated acids for sample recovery followed by laborious sample clean-up, preconcentration, and long separation time. LC–MS/MS methods have also been reported for the analysis of PME in human plasma and urine [26,30]. These methods are unsatisfactory since they require a long and expensive procedure of SPE, consumption and injection of a large volume of samples (100–200 μL), and long separation time with poor chromatography. The reported sensitivity for PME in any matrices ranges from 30 to 300 ng/mL (g) per 10 μL injection.

Another class of antibiotic peptide drugs is glycopeptide antibiotics. This class is composed of glycosylated cyclic or polycyclic nonribosomal peptides, neutral sugars and an amino sugar. The peptides consist of cross-linked unusual aromatic amino acids and convention amino acids such as aspartic acid [31]. Significant glycopeptide antibiotics include VCM, dalbavancin, teicoplanin, telavancin, bleomycin, ramoplanin, and decaplanin [32,33]. They are soluble in aqueous solvent but not in nonpolar organic solvents. This class of drugs inhibits the synthesis of cell walls in susceptible microbes by inhibiting peptidoglycan synthesis. They bind to the amino acids within the cell wall, preventing the addition of new units to the peptidoglycan.

VCM is a benchmark compound for various preclinical pharmacology models treating endocarditis [34,35]. However, the unique molecular properties of VCM presented similar bioanalytical challenges as PMXs. Current LC–MS methods include using strong cation exchange SPE for sample preparation from serum followed by LC-full scan Fourier transform MS [36], online sample extraction followed by column switching technique [37], and an offline sample extraction technique using TFA and methanol [38]. The LLOQ obtained ranged from 1 to 10 ng/mL. The above methods require complicated extraction procedure, large injection volume and long separation time. Moreover, the previous studies did not apply sample recovery optimization.

The two classes of antibiotics, lipopeptide PMXs and glycopeptide VCM, actually have similarities. They are both peptide drugs of similar molecular weight range; the sizes of the peptide parts are dominant in either the lipopeptide molecules or in the glycopeptide molecule. The purpose of the study is to develop and validate a general bioanalytical method based on the same principle for the above antibacterial peptide compounds.

2. Experimental

2.1. Chemicals and reagents

All solvents used were of HPLC grade and purchased from Fisher Scientific (Pittsburgh, PA, USA). Formic acid (88%) was supplied by J. T. Baker (Phillipsberg, NJ, USA). TCA (99+%) was purchased from Acros (Morris Plains, NJ, USA). Control Rat Plasma in EDTA K2 (Individual MALE 031-APEK2-MI) was purchased from Bichemed (Wichester, MA, USA). PMB (polymyxin B sulfate), PME (colistin methanesulfonate), VCM and dalbavancin were obtained from Pfizer Global Research & Development (Groton, CT, USA). [Glu[1]]-Fibrinopeptide B human (≥97%) was purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Equipment

A standard multitube vortex-mixer from VWR Scientific Products (West Chester, PA, USA) was used for vortex-mixing, and an Eppendorf centrifuge model 5810R from Brinkmann Instruments Inc. (Westbury, NY, USA) was used for centrifugation. An Applied Biosystems/MDS Sciex (Concord, ON, Canada) model API 4000 triple quadrupole mass spectrometer equipped with a Shimadzu LC-10AD Prominence solvent delivery system, degasser and SCL-10 Avp system controller (Columnbia, MD, USA) was used for LC–MS/MS analysis. A Leap Technologies CTC PAL autosampler with Shimadzu 10AD pump was used. A Harvard Apparatus (South Natick, MA, USA) syringe pump with a 500 μL syringe from Hamilton Co. (Reno, NE, USA) was employed for compound infusion.

2.3. Sample preparation using TCA or acetonitrile (ACN) induced plasma protein precipitation

For TCA induced plasma precipitation, TCA was diluted in water to obtain 0.1%, 1%, 5%, 10%, 15%, 20%, 25%, 30% and 35% concentration (w/v). To 50 μL plasma samples, 30 μL TCA at various concentrations were added; white protein precipitation was observed; then 170 μL water was added. Samples were centrifuged at 4000 rpm for 5 min, and 50 μL of the supernatants were aliquoted into a 1.2 mL polypropylene 96-well plates for sample analysis. For ACN induced plasma precipitation, to 50 μL plasma samples, 25–200 μL ACN (at various ACN/water ratios) were added to 50 μL of plasma samples, and protein precipitation was observed. 50 μL of the supernatants were aliquoted and reconstituted in 10% ACN for sample analysis. Analyte recovery was calculated by peak areas count ratios of samples recovered from plasma and samples from water solutions at correspondent TCA concentrations. Samples at each concentration level were analyzed in triplicate over three independent batch runs.

2.4. Preparation of calibration standards

Stock solutions of PMB (containing PMB1 and PMB2), PME (containing PME1 and PME2), Fibrinopeptide B, VCM and dalbavancin were prepared as 1 mg/mL concentration in water with their purity factors considered. Fibrinopeptide B and dalbavancin were further diluted to 500 ng/mL for use as internal standards for PMXs and VCM, respectively. Stock solutions were serially diluted with rat plasma or water. Analytical standards used to construct calibration curves were prepared separately for each type of extraction method. The stock solutions of the compounds were prepared in water and the stock standard solutions were carried out by serial dilutions of the stock solutions to desired concentrations. Plasma and neat solvent working standards were prepared by spiking known quantities of the stock standard solutions to the blank rat plasma and water, respectively. The final concentrations for PMXs