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High-throughput determination of vancomycin in human plasma by a cost-effective system of two-dimensional liquid chromatography

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ABSTRACT

Therapeutic drug monitoring (TDM) is one of the most important services of clinical laboratories. Two main techniques are commonly used: the immunoassay and chromatography method. We have developed a cost-effective system of two-dimensional liquid chromatography with ultraviolet detection (2D-LC-UV) for high-throughput determination of vancomycin in human plasma that combines the automation and low start-up costs of the immunoassay with the high selectivity and sensitivity of the liquid chromatography coupled with mass spectrometric detection without incurring their disadvantages, achieving high cost-effectiveness. This 2D-LC system offers a large volume injection to provide sufficient sensitivity and uses simulated gradient peak compression technology to control peak broadening and to improve peak shape. A middle column was added to reduce the analysis cycle time and make it suitable for high-throughput routine clinical assays. The analysis cycle time was 4 min and the peak width was 0.8 min. Compared with other chromatographic methods that have been developed, the analysis cycle time and peak width for vancomycin was reduced significantly. The lower limit of quantification was 0.20 μg/mL for vancomycin, which is the same as certain LC–MS/MS methods that have been recently developed and validated. The method is rapid, automated, and low-cost and has high selectivity and sensitivity for the quantification of vancomycin in human plasma, thus making it well-suited for use in hospital clinical laboratories.

1. Introduction

Clinical laboratory services are at the heart of modern health care and have a great influence on clinical decision-making. Overall, 60–70% of the most important decisions concerning admission, discharge, and medication use are based on laboratory test results [1]. TDM is one of the most important services of clinical laboratories and is based on pharmacokinetics principles. TDM involves the determination of drug concentrations in plasma to adjust individual doses, optimize clinical treatments, avoid toxicity for drugs with a narrow therapeutic window, and achieve safe, effective, and rational drug use. However, therapeutic drug monitoring in clinics requires: (1) high analytical selectivity and sensitivity because many types of biological samples, such as plasma, serum, urine, and saliva, contain complex matrices, and the drug concentrations tend to be low; (2) clinical routine assays developed for high sample throughput with a short turnaround time; and (3) relatively inexpensive analytical tools and low-cost per-sample analytical methods, because cost considerations have become the primary issue for clinical laboratories in hospitals in an age of cost-effective health care; the need to provide a financial justification when selecting analytical methods is widely acknowledged [2–4].

Vancomycin is a glycopeptide antibiotic with strong bactericidal activity against Gram-positive bacteria. Many immunoassay and chromatography methods for the quantification of vancomycin in biological fluids have been developed [5–21]. However, these methods have many limitations and cannot completely meet the three key requirements mentioned above. Immunoassays lack specificity and sensitivity; studies have found that vancomycin degradation products interfere with certain immunoassays, and immunoassays have a quantification limit of 2 μg/mL [5,6]. Meanwhile, most chromatography methods that use UV detection, fluorescence detection or mass spectrometric detection require a lengthy analysis cycle time of more than 7.5 min per sample (most require 10 min) [7–18,21], which makes them unable to meet the clinical requirements for testing highly varied and high-throughput samples. Most importantly, because vancomycin has a high molecular weight as well as a number of functional groups that interact with the stationary and mobile phases, a wide peak width (peak

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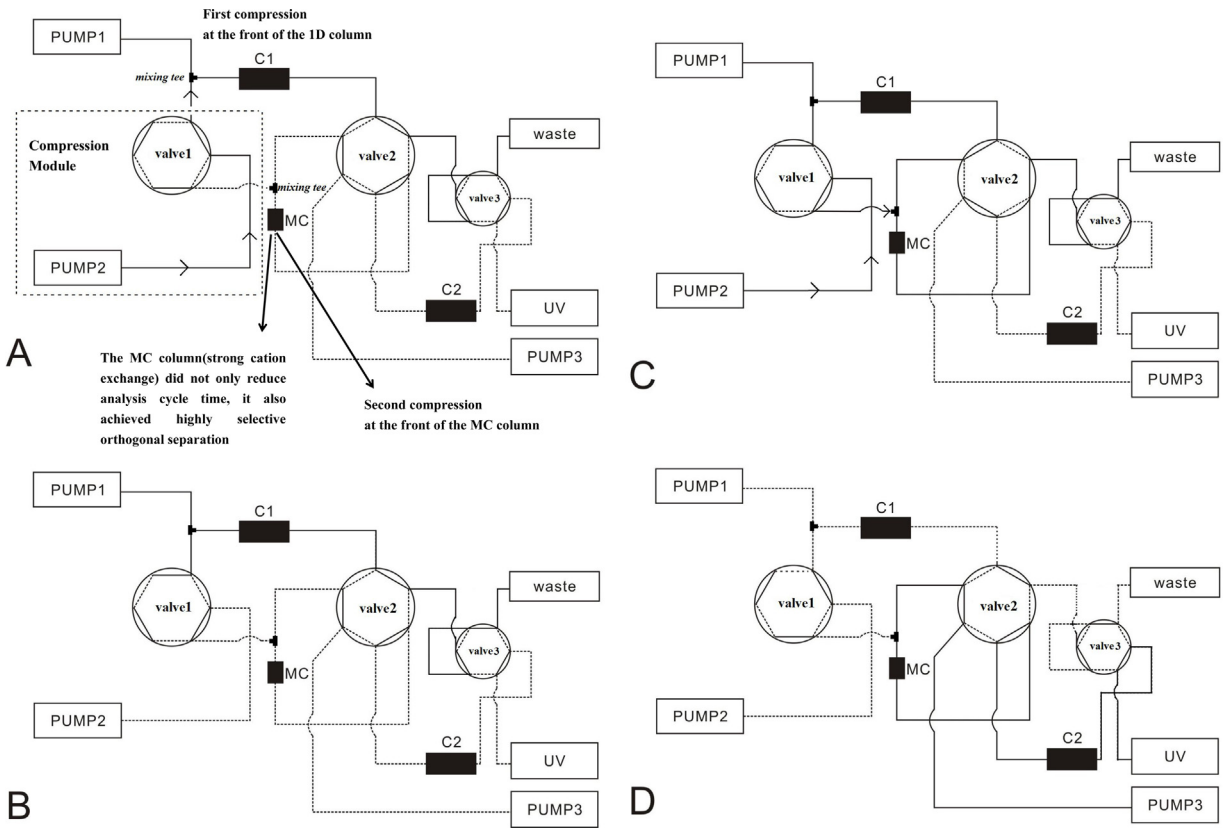

Fig. 1. Schematic diagram of two-dimensional liquid chromatography system. The black solid line indicates the analytes was eluted at the current flow. C1 – first-dimension column (RP column); MC – middle column (SCX column); C2 – second-dimension column (RP column). This figure indicates the initial positions of the three valves, which were 0, 0, and 0, respectively. (A) While the sample was injected via the autosampler, PUMP2 was started (valve1, valve2, valve3 = 0, 0, 0). (B) The sample was separated on the first-dimension column (valve1, valve2, valve3 = 1, 0, 0). (C) Rotation of the six-port valve connected the first-dimension and middle columns, and the analytes were transferred to the middle column. PUMP2 was restarted when the heart-cut window started (valve1, valve2, valve3 = 1, 1, 0). (D) The middle column and second-dimension column were connected by rotating the six-port valve. The mobile phase of PUMP3 eluted the analytes trapped on the middle column, transferring it to the second-dimension column (valve1, valve2, valve3 = 0, 0, 0).

widths greater than 1.2 min with tailing or distortion) can often be observed [7–11,13–19], with some studies reporting peak widths greater than 2 min [17,19]; this causes errors in integration at low concentrations as well as a poorly resolved vancomycin peak that is without sufficient selectivity. Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) has been the preferred method for vancomycin determination in complex biological matrices [18–21], but it has many limitations, one of which is the high initial investment and high maintenance costs that limits its availability to a small number of clinical laboratories for TDM in certain countries [22,23]. In recent years, with the development of ultra-high performance liquid chromatography (UPLC) and sub-2 μm particle packed in columns, it is possible to attain better peak capacity and analysis cycle time, and several UPLC–MS/MS methods have been reported [20,21]. Shou et al. [21] was completed within 10 min, while Tsai et al. [20] achieved an analysis cycle time of 4 min. Our analysis cycle time of 2D-LC method was 7.5 min, which, although faster than that of most chromatography methods [7–18,21], is still slower than that of UPLC–MS/MS. Thus, we developed a high-throughput mode for R&D work with a cycle time of 4 min, equivalent to the UPLC coupled with triple quadrupole MS method reported by Tsai et al. [21] and our costs are far lower than that of UPLC–MS/MS. Moreover, to reduce ionization matrix effects, samples must first be prepared, and certain sample preparation methods (liquid–liquid extraction and off-line solid-phase extraction) are very time-consuming and labor-intensive [24].

In order to overcome the weaknesses of methods in existing literature and meet the three key requirements mentioned above, we established a cost-effective system of two-dimensional liquid chromatography with ultraviolet detection (2D-LC-UV) for the high-throughput determination of vancomycin in human plasma. The 2D-LC combines the automation and low start-up costs of the immunoassay method with the high selectivity and sensitivity of the liquid chromatography coupled with mass spectrometric detection to fulfill the three key requirements mentioned above.

2. Materials and methods

2.1. Materials

Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Ammonium acetate, ethylene glycol, and trichloroacetic acid were purchased from Sinopharm Chemical Reagent Limited, Co. (Shanghai, China). Vancomycin was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All solvents used were HPLC grade and all other chemicals were analytical grade. Blank human plasma was obtained from the Changsha Blood Donor Service (Changsha, China). Deionized water was generated using a Millipore Milli-Q system (Bedford, MA, USA).

2.2. Standard solutions and calibration curve

Standard stock solutions of vancomycin were prepared in methanol at a concentration of 1 mg/mL. Working standard solutions of vancomycin were prepared by diluting the stock solution