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High-performance liquid chromatography assay with programmed flow elution for cisatracurium in human plasma: Application to pharmacokinetics in infants and children

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ABSTRACT

A high-performance liquid chromatography (HPLC) assay with fluorescence detection (FLD) for quantification of cisatracurium in human plasma was developed and fully validated. Liquid–liquid extraction was employed for sample preparation. The separation was carried out on a C_{18} column with ternary mobile phase composed of 30 mmol L^{-1} phosphate buffer (pH 3.0), acetonitrile and methanol (60:35:5, v/v/v). Verapamil was used as the internal standard. The isocratic elution with programmed flow rate was employed by setting at 0.8 mL min^{-1} from 0 to 3.5 min, 0.5 mL min^{-1} from 3.5 to 6 min, and 1.0 mL min^{-1} from 6 to 10 min. The fluorescence detection was performed at 236 nm for excitation and 324 nm for emission. The assay was linear from 50 to 2800 ng mL^{-1}, with a detection limit of 12 ng mL^{-1}. The correlation coefficient (r) for linear regression was 0.9997. The intra-day coefficients of variation (CVs) were less than 2.0%, and the inter-day CVs were less than 4.0%. The mean recoveries were in the range of 92.1–100.4%. The total HPLC run time was less than 10 min. The developed HPLC method was fast, simple, sensitive, accurate and suitable for studying the pharmacokinetics of cisatracurium in infants and children after intravenous administration.

1. Introduction

Cisatracurium is an intermediate-acting, non-depolarizing muscle relaxant which has been used adjunctively in anesthesia to provide the relaxation of skeletal muscle and to facilitate endotracheal intubation during surgery [1–4]. Compared with other muscle relaxants and concomitant drugs [5–8], cisatracurium possesses better hemodynamic stability and lower histamine releasing potential [9,10]. At physiological pH and temperature, cisatracurium could degrade in plasma by an organ-independent Hofmann elimination [11–13].

In recent years, the plasma pharmacokinetics of cisatracurium has been described in a variety of patient populations with the aim of establishing appropriate dosing schedules [14–20], but the methods designed for determination of cisatracurium concentrations in plasma are scarce [21]. In 2004, Sayer et al. [21] reported a liquid chromatography–electrospray ionisation-mass spectrometry (LC-ESI-MS) technique to determine cisatracurium in human plasma. Because the procedure was expensive, it did not become a common assay for clinical application. In 2010, Błażewicz et al. [22] proposed a liquid chromatography method with charged aerosol detection (LC-CAD) for simultaneous quantification of cisatracurium and another two muscle relaxants. In their work, they focused on the analysis in pharmaceutical preparations, so the application in plasma samples could not be discussed. Later, Imbeault et al. [14] and Withington et al. [15] investigated the plasma pharmacokinetics of cisatracurium in children and determined the plasma concentrations of cisatracurium using published HPLC-FLD method by Bryant et al. for urine samples [23]. However, the analysis time of the method (34.5 min) was relatively long. In 2012, Liu et al. [16] evaluated the plasma pharmacokinetics of cisatracurium in critically ill patients with severe sepsis. In their work, the plasma concentrations of cisatracurium were determined using published HPLC-FLD method for atracurium [24], which is a stereoisomer of cisatracurium.

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Since there is no method suitable for the quantitative analysis of cisatracurium in plasma, we are interested in developing an analytical method to determine the plasma concentrations of cisatracurium and to facilitate the investigation of its plasma pharmacokinetics. In the present study, we have developed an HPLC-FLD method for determination of cisatracurium in human plasma. Small volumes of plasma sample were required to facilitate infants and children patients. Sufficient separation of the analytes was achieved by using ternary mobile phase and programmed flow elution in the relatively short time. The presented method has proved suitable for the pharmacokinetic investigation of cisatracurium in infants and children.

2. Experimental

2.1. Chemicals and reagents

Cisatracurium and Verapamil (internal standard, IS) were purchased from the National Institutes for Food and Drug Control (Beijing, China). Methanol and acetonitrile were of chromatographic grade and obtained from Tedia (Fairfield, OH, USA). HPLC-grade dichloromethane was obtained from Guangfu Fine Chemical Industry Research Institute (Tianjin, China). Isopropanol was of analytical grade and purchased from Chuandong Chemical Co., Ltd. (Chongqing, China). Picric acid was of analytical grade and purchased from Laimei Pharmaceutical Co., Ltd. (Chongqing, China). HPLC-grade water was purified with a MilliQ^{TM} System (Millipore, Milford, MA, USA).

2.2. Chromatographic system and conditions

The HPLC equipment was Agilent LC-1100 equipped with fluorescence detector, quaternary pump and vacuum degasser (Palo Alto, CA, USA). The analytical column (Shim-pack VP-ODS; 150 mm × 4.6 mm; 5 μm) and the guard column (Phenomenex C_{18}; 4 mm × 3 mm; 5 μm) were used for the analysis at 25 °C. Separation was carried out using the mobile phase consisting of 30 mmol L^{-1} PBS (pH 3.0), acetonitrile and methanol (60:35:5, v/v/v). The programmed flow rate was set at 0.8 mL min^{-1} from 0 to 3.5 min, 0.5 mL min^{-1} from 3.5 to 6 min and 1.0 mL min^{-1} from 6 to 10 min. The eluates were monitored at 236 nm for excitation and 324 nm for emission.

2.3. Preparation of calibrators

Standard stock solutions of cisatracurium and IS were prepared with HPLC-grade water at the concentrations of 500 μg mL^{-1} and 100 μg mL^{-1}, respectively, and stored at −20 °C. The working solutions of IS were further diluted in HPLC-grade water at the concentrations of 350 ng mL^{-1} before use. The working solutions of cisatracurium were freshly prepared with HPLC-grade water for a set of calibrating standards at 50, 100, 200, 400, 800, 1600, 2400 and 2800 ng mL^{-1} before use.

2.4. Preparation of samples

To a 5 mL centrifuge tube, 0.25 mL of acidified plasma sample (see Section 2.6 for details on acidification), 0.25 mL of IS and 0.25 mL of picric acid solution were added and vortex-mixed for 10 s. Then the sample was treated with 2.5 mL of the mixture of dichloromethane and isopropanol (85:15, v/v), agitated for 15 min at 120 r min^{-1} and centrifuged for 10 min at 3000 × *g*. The organic extract was transferred to another 5 mL centrifuge tube and evaporated to dryness under a nitrogen stream at 30 °C. The residue was dissolved in 100 μL of methanol-water (40:60, v/v), centrifuged at 15,000 × *g* for 5 min at 4 °C. Sixty microliters of samples were injected using full loop injection method (20 μL loop) to ensure that 20 μL of each sample was introduced into the HPLC system.

2.5. Method validation

2.5.1. Calibration curve and linearity

The calibration samples were prepared by adding 20 μL of a series of standard working solutions of cisatracurium into 230 μL of mixed drug-free plasma, and then performed as sample-preparation described above. Eight-point calibration curves ranging from 50 to 2800 ng mL^{-1} were constructed in triplicate by plotting the peak-area ratio of cisatracurium over IS (*Y*) versus the concentration of cisatracurium (*X*) in the calibration samples. Linearity was generated by least-squares linear regression of the calibration curve. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated with a signal-to-noise (S/N) ratio of 3:1 and 10:1, respectively.

2.5.2. Accuracy and precision

Twenty microliters of standard working solutions of low (150 ng mL^{-1}), middle (800 ng mL^{-1}) and high concentration (2400 ng mL^{-1}) were added into 230 μL of mixed drug-free plasma and then performed as sample-preparation described previously. The intra-day accuracy and precision were determined by analyzing five replicates of the spiked plasma sample at three concentration levels on the same day. The inter-day accuracy and precision was determined by analyzing the spiked plasma sample on five consecutive days. The accuracy was expressed as the relative error of nominal concentration (RE, in percent), while the precision was given by the intra-day and inter-day coefficients of variation (CV, in percent).

2.5.3. Extraction recovery

The extraction recovery was evaluated using spiked plasma samples at low (100 ng mL^{-1}), middle (800 ng mL^{-1}) and high concentration (2400 ng mL^{-1}) for cisatracurium. These samples were treated as sample-preparation described previously. The recovery was expressed as [detected concentration/spiked concentration] × 100%.

2.5.4. Stability

The stability of cisatracurium in plasma samples before preparation was assessed at −20 °C after 0, 10, 20 and 40 days of storage. Prior to analysis, samples were brought to room temperature and vortex-mixed.

The stability of cisatracurium in plasma samples after preparation was assessed after 0, 4, 9 and 24 h of storage without light exposure at room temperature.

2.5.5. Selectivity

Ten drug-free plasma samples from different healthy subjects were tested to demonstrate the selectivity of the method. The retention times of endogenous compounds in the matrix were compared with those of cisatracurium and IS.

In addition, a study of potential interferences in the chromatographic analysis of cisatracurium was performed by selecting the drugs (propofol, midazolam, sufentanil, sevoflurane, and penehyclidine hydrochloride) concomitantly used by the patients in this study.

2.6. Pharmacokinetic study

Six subjects, aged 6 months to 4 years, undergoing surgery for congenital harelip or cleft palate repair were from Children's Hospital of Chongqing Medical University. Subjects with clinical evidence of neuromuscular, hepatic, renal or pulmonary disease