

Determination of Rocuronium in Human Plasma by High Performance Liquid Chromatography-Tandem Mass Spectrometry and its Pharmacokinetics in Patients

Yuan Guiyan, Zhang Rui, Wang Benjie, Wei Chunmin, Liu Xiaoyan, Zhao Wenjing and Guo Ruichen*

Institute of Clinical Pharmacology, Qilu Hospital of Shandong University, Jinan Shandong, China

Abstract

A sensitive and selective high performance liquid chromatography-tandem mass spectrometric (HPLC-MS/MS) method was developed and validated for the determination of rocuronium in human plasma. The plasma samples were separated on an Agilent SB-C₁₈ column (150×2.1 mm, 3.5 μm) with a mobile phase consisted of 20 mM ammonium acetate/methanol/ acetonitrile (20/40/40, v/v/v) at a flow rate of 0.7 mL·min⁻¹. Electrospray ionization (ESI) source was applied and operated in the positive ion mode. Multiple Reaction Monitoring (MRM) modes with the transitions of m/z 529.3→487.3 (rocuronium) and 321.0→275.0 (IS) was used. A good linearity was obtained in the concentration range of 5~3000 μg·L⁻¹ (r=0.9976, n=5). The inter- and intra-day precision (RSD) were less than 8.1%. The extraction recoveries were 92.0~92.6%. Rocuronium in plasma was stable when frozen at -20°C for 24 hours and seven days, and also stable after two freeze-thawing cycles. The method is simple, quick, sensitive, reproducible and can be used for the pharmacokinetic and bioequivalence study of rocuronium.

Keywords: Rocuronium; High performance liquid chromatography-tandem mass spectrometry; Pharmacokinetics

Introduction

Rocuronium, a new type of muscle relaxant with the features of single quaternary ammonium steroids, medium aging and non depolarizing, was used for anesthesia and surgery of endotracheal intubation of muscle relaxation, which has quick effect, short duration and no cumulative effect, and do not produce tachycardia and blood pressure change, no histamine release, and other features. It is one of the most widely used muscle relaxants at present [1,2].

Less studies were reported on the determination of rocuronium. Yu et al. [3] reported a gas chromatography mass spectrometric (GC-MS) method for determination rocuronium in human plasma, which has higher limit of quantitation and longer time for sample analysis. Shi et al. [4] developed a high performance liquid chromatography with fluorescence detection (HPLC-FD) method to determine the concentration of rocuronium in patient's plasma, which sample treatment was time-consuming because of rocuronium derivatization. Farenc et al. [5] established a high performance liquid chromatography-mass spectrometric (LC-MS) method to determine the concentration of rocuronium in human plasma. In this method, the limit of quantitation is higher (25 ng·mL⁻¹), and trifluoroacetic acid was added to the mobile phase which can damage the ion source because it is less volatile. The aims of this paper were to establish a LC-MS/MS method to determine rocuronium in human plasma and to study the pharmacokinetics of rocuronium in patients used the established method.

Experimental

Chemical, reagents and instruments

Rocuronium standard (purity>99%, Batch number 63254132) was provided by Organon Pharmaceutical Co Ltd (Oss, Netherlands). Lorazepam standard (Batch number 171253-200401) was provided by national institutes for food and control. HPLC grade methanol and acetonitrile (Batch number LOTG06E10 and LOTE30818) were purchased from J.T. Baker Company (USA). Analytical pure ammonium acetate (Batch number 060223) was provided by Chemical Industry Institute of Shandong Province (Jinan, China).

The HPLC components consisted of an Agilent 1200 liquid chromatography system with a G1312B binary pump, G1379B degasser, G1316B thermostated column compartment and G1367C auto-sampler (Agilent Technologies, USA). An Agilent 6410 triple quadrupole mass spectrometer equipped with a Turbo Ion Spray (ESI) source was used for mass analysis and detection (Agilent Technologies, USA).

Chromatographic conditions

Chromatographic separation was performed using a Agilent SB C₁₈ column (3.5 μm particle size, 150×2.1 mm internal diameter; Agilent Technologies). The isocratic mobile phase consisted of methanol/ acetonitrile/20 mM ammonium acetate buffer solution (20/40/40, v/v/v). The flow rate of the mobile phase and the column oven temperature were set at 0.7 mL/min and 20°C, respectively.

Mass spectrometric conditions

The ESI source was set to positive ion mode and multiple reaction monitoring (MRM) mode was used to detect rocuronium and lorazepam (internal standard, IS) at the transition of 529.3→487.3 and 321.0→275.0, with spray gas pressure of 40 psi, protective air of nitrogen gas at a flow rate of 9 L/min, capillary voltage of 4000 V, fragment electric voltage of 100 V, and collision energy of 45 eV for rocuronium and 20 eV for I.S.

*Corresponding author: Guo Ruichen, Institute of Clinical Pharmacology, Qilu Hospital of Shandong University, Jinan Shandong, China, Tel: (0351)82169636; Fax: (0531)86109975; E-mail: grc7636@126.com

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Sample preparation

A 25 μL aliquot of the I.S. solution (10 $\mu\text{g}/\text{mL}$) and 300 μL mixture of methanol/acetonitrile (1/1, v/v) were added to 200 μL of plasma samples, mixed on a Multi-Tube Vortexer VX-02 (TARGIN TECH CO., Ltd) for 1 minute, followed by 5 minutes centrifugation at 11132x g. A 5 μL of upper organic layer was injected for HPLC/MS/MS analysis.

Pharmacokinetic study design

Seven patients, who will undergo a surgery using rocuronium as anesthetic, signed the informed consent form after understanding the content, rights, obligations and risks. 4 mL blood samples were collected in heparinized tubes before (0) and 2, 4, 9, 15, 25, 40, 60, 90, 120, 180, 240, and 360 min after hocused. Blood samples were centrifuged at 1752x g for 10 minutes and plasma was subsequently quoted and stored at -20°C until analyzed. Drug and Statistic (DAS, version 2.0, by Chen et al. [6], China) was used to calculate the main pharmacokinetic parameters as half life ($t_{1/2}$) and area under the plasma concentration versus time curve (AUC_{0-24} and $\text{AUC}_{0-\infty}$). The peak plasma concentration (C_{max}) and its corresponding time (T_{max}) were observed values.

Results

Method validation

Specificity was assessed by analyzing six different human blank plasma samples.

Typical chromatograms are shown in Figure 1. Matrix effects were evaluated by signal response comparison of six extracted blank plasma samples with those of six analytical standards (at three concentration levels of QCs). No matrix effects were detected in the study.

The calibration curves were constructed by analyzing five independent standard plasma samples. The peak area ratios of rocuronium and IS were measured and plotted against the concentrations of rocuronium spiked with blank plasma, and the least square method with $1/x^2$ weighting factor was used. The weighted regression equation was $y=12.2106x+0.0354$ with a correlation coefficient r^2 of 0.9976. It was found to be linear over the range of 5-3000 $\mu\text{g}/\text{mL}$.

The extraction recoveries of rocuronium were determined by comparing the mean peak areas of the extracted QC samples at 3 concentrations of 10, 100, 2000 $\mu\text{g}/\text{mL}$ with mean peak areas of appropriate concentrations of standard solutions. The results were found to be 92.0, 92.9 and 92.6% with a precision (RSD) of 1.68, 0.71 and 1.22%, respectively.

Precision and accuracy were assessed investigating QC samples on three different validation days. The intra-day precision (RSD) ranged between 1.10 and 1.95%, while the inter-day precision (RSD) ranged between 3.97 and 8.05%.

The freeze-thaw and freeze stabilities of rocuronium were evaluated by analyzing QC samples undergoing two freeze-thaw cycles (-20°C to room temperature) and placing QC samples at -20°C for 24 h and 7 days. The stability results suggested that rocuronium was stable under these storage and processing conditions.

Pharmacokinetic study

The concentration of rocuronium in patient's plasma was determined using the established LC-MS/MS method. The main pharmacokinetic parameters of rocuronium were as follows: $t_{1/2}=78.18 \pm 26.88$ min, $T_{\text{max}}=2.29 \pm 0.76$ min, $C_{\text{max}}=27.99 \pm 48.93$ $\mu\text{g}/\text{mL}$, $\text{AUC}_{0-360}=276.93 \pm 220.56$ $\mu\text{g}/\text{mL}\cdot\text{h}$, $\text{AUC}_{0-\infty}=279.67 \pm 220.29$ $\mu\text{g}/\text{mL}\cdot\text{h}$.

Conclusion

A sensitive and selective LC-MS method was developed and validated for the determination of rocuronium in human plasma. This has been successfully applied to pharmacokinetic studies in patients who were hocused using rocuronium.

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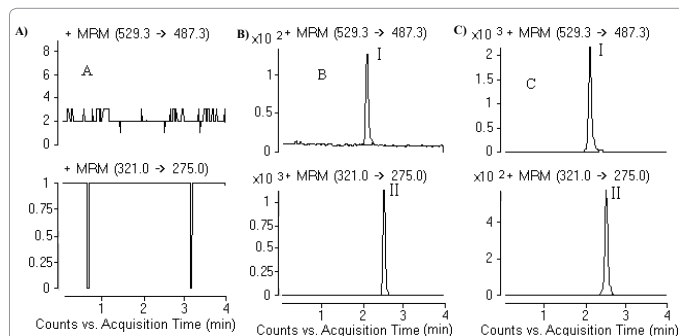


Figure 1: a) Chromatograms of blank plasma. b) Plasma spiked with rocuronium and IS standard, c) Extracted plasma sample from a patient spiked with IS (α -rocuronium, α -IS).