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Development of a Novel Method to Measure Ketanserin in Guinea Pig Plasma by Multiple Reaction Monitoring and its Application to Pharmacokinetic Studies

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***Abstract*—Ketanserin is a 5-HT2A receptor antagonist that is effective at lowering blood pressure in hypertension and inhibiting platelet aggregation. For this reason, it is commonly used to confirm 5-HT2A receptor-meditated events and can be used as a radioligand to evaluate 5-HT2A receptor distribution. Recently, ketanserin has been studied for its novel usages, such as relieving inflammatory pain in combinations with other drugs. Previous methods to quantify ketanserin have relied on ultraviolet detection and fluorescence detection. Herein we report the development of a novel method that utilizes liquid chromatography tandem mass spectrometry and multiple reaction monitoring to simultaneously determine the concentration of ketanserin in guinea pig plasma. This method uses an isocratic mobile phase of 90% acetonitrile at a flow rate of 0.4 mL/min to separate plasma prior to infusion on the mass spectrometer. Instrument settings were optimized for single charged precursor ions, (M+H)+, and domperidone was used as an internal standard at 500 ng/mL. The lower limit of quantification of ketanserin in guinea pig plasma was 1 ng/mL. Specificity, accuracy, precision, recovery, matrix effects, and stability of the method were well within Food and Drug Administration guidelines for the validation of bioanalytical methods. As a proof-of-principle, this validated method was successfully applied to a pharmacokinetic study in guinea pigs after intravenous administration of 3mg/kg ketanserin. This simple, rapid, and accurate method is a better prospect for quantifying ketanserin than previously available methods.**

Keywords—Ketanserin, HPLC-MS/MS, Pharmacokinetics, Guinea pig,

I. INTRODUCTION

Ketanserin, 3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1H-quinazoline-2,4-dione, is a 5-HT2A receptor antagonist with weak adrenergic receptor binding properties that is effective at lowering blood pressure in essential hypertension and also inhibits platelet aggregation [1,2]. It is especially useful as an antagonist to confirm 5-HT2A receptor-meditated events such as a sudden edematous response in soft tissues [3] and it has been used as a radioligand for 5-HT2A to analyze serotonin-2A receptor distribution in the human brain [4]. Recent studies have focused on discovering novel uses for ketanserin such as inflammatory pain relief in combination with other drugs [5].

Previous methods to quantify ketanserin have utilized high-performance liquid chromatography (HPLC) ultraviolet detection and fluorescence detection [6]. These methods have some limitations with regard to efficacy for analysis due to complicated liquid-liquid extractions used for sample preparation, long run times, typically high limits of quantification, and relatively large sample volumes. Conversely, there have been no studies of methods for simultaneous determination of ketanserin by liquid chromatography tandem mass spectrometry (LC-MS/MS) and multiple-reaction monitoring (MRM).

The results of the current study describe the performance of a novel LC-MS/MS MRM-based method for the simultaneous determination of ketanserin in guinea pig plasma and apply this method to a pharmacokinetic study of ketanserin in guinea pigs. These results demonstrate the performance of the method as well as its potential to provide highly accurate ketanserin measurements.

II. METHOD

A. *Materials and reagents*

Ketanserin was purchased from Santa Cruz Biotechnology (Dallas, TX, USA) and the internal standard (IS) domperidone was from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol and acetonitrile (ACN) were attained from Merck (Darmstadt, Germany).

B. *Sample preparation*

Stock solutions of ketanserin were serially diluted with ACN from an initial concentration of 1 mg/mL. Calibration and quality-control (QC) samples were prepared by adding 5 µL of these ketanserin standard solutions to 45 µL of drug-free plasma. Plasma samples containing ketanserin at 1 ng/mL, 10 ng/mL, 100 ng/mL, 1 µg/mL, and 5 µg/mL were analyzed and used to construct a calibration curve. The lower limit of quantification (LLOQ), low, medium, and high QC concentrations of ketanserin were 1 ng/mL, 10 ng/mL, 1000 ng/mL, and 5,000 ng/mL, respectively. A 500 ng/mL domperidone IS stock was prepared by dissolving domperidone in ACN. The samples were vortexed for 5 min and centrifuged at 13,200 rpm for 10 min. Then, a 5 µL aliquot of the resulting supernatant was injected onto the LC.

C. LC-MS/MS analysis

To optimize MS/MS conditions, a solution containing 100 ng/mL of ketansrin was infused into the mass spectrometer (API 2000; Applied Biosystems, Foster City, CA, USA) at a flow rate of 10 µL/min. The turbo ion spray interface was operated in positive ion mode at a spray voltage of 5,500 V and an ion spray temperature of 350°C. Instrument parameters were optimized such that the precursor ions for the analytes and internal standard were single charged molecular ions, (M+H)+. The declustering potential, entrance potential and collision energy for ketanserin were 117 V, 10 V and 36.5 V, respectively, compared to 110 V, 10 V and 38.9 V, respectively, for the IS. Ketanserin was quantified by multiple reaction monitoring using a peak-area ratio method with the IS.

Separation prior to infusion into the mass spectrometer was performed on a model 1100 HPLC system (Agilent Technologies, Inc., Santa Clara, CA, USA). A Gemini column (50 × 4.6 mm, 5 µm; Phenomenex, USA) was used with an isocratic mobile phase of 90% ACN at a flow rate of 0.4 mL/min. The column oven and auto sampler were maintained at 40°C and 4°C, respectively. The total analytical run time was 3 min. The Analyst® software (ver. 1.4.2; Applied Biosystems) was used for LC-MS/MS system control and analytical data processing.

D. Method validation

The specificity of the method was evaluated using guinea pig blank plasma samples obtained fresh from five animals. The intra- and inter-day precision and accuracy were estimated by analyzing the QC samples. The limits of precision and accuracy for acceptable data were within 15% or 20% at the LLOQ. Recovery was calculated by comparing the mean peak area of ketanserin in plasma samples spiked before protein precipitation with the peak areas in samples spiked after protein precipitation. Matrix effects were evaluated by comparing the mean peak area of ketanserin in plasma samples spiked before protein precipitation with the peak area of ketanserin added directly to the mobile phase. Short-term (6 hours), long-term (14 days), freeze-thaw, post-preparative, and stock stability tests were assessed at low and high QC concentrations.

E. Animals

This study was approved by the Ethics Committee of Animal Experimentation of Chungnam National University. Five Guinea pigs (360 to 380 g, male) were housed under temperature-controlled conditions (21°C) with a 12 hour light-dark cycle. The animals were given free access to food and water prior to experimentation, and an intravenous dose of ketanserin was given after an overnight fast.

F. Study design

The animals were fasted overnight prior to single dosing. Ketanserin at 3 mg/kg was administered intravenously. Blood samples were collected from the jugular vein 5 min, 10 min, 20 min, 25 min, 30 min, 1 h, 2 h, and 4 h after administration of ketanserin. Each collected blood sample was centrifuged at 13,200 rpm for 10 min. Plasma was separated and stored at -70°C until analysis. Noncompartmental analysis was performed using Phoenix (Pharsight, Basel, Switzerland) to obtain pharmacokinetic parameters (Cmax, t1/2, AUCt).

III. RESULTS AND DISCUSSION

A. Multiple reaction monitoring

The precursor-product ion transitions between Q1 and Q3 for ketanserin and the IS were 395.7 → 189 m/z and 425.5 → 175 m/z, respectively (Figure 1). The retention times for ketanserin and the IS were 1.54 min and 1.37 min, respectively. By evaluating the chromatogram of a blank plasma sample analyzed by the described method it was evident that none of the matrix components interfered with the analytes or the IS at their corresponding retention times (Figure 2). The retention times of ketanserin and IS were reproducible throughout the study and no column deterioration was observed.

B. Method validation

The calibration curve was linear from 1 to 5,000 ng/mL ketanserin. Using linear regression analysis with a weighting of 1/y2, the calibration curve equation was y = 0.00254x + 0.00224 with an r2 > 0.99. Intra-day and inter-day precision and accuracy of the QC samples in guinea pig plasma are shown in Table 1. The accuracy (relative error, %RE) of ketanserin ranged between -12.8% and 13.2%, and the precision (coefficient of variation, %CV) was between 1.5% and 16.3%. The precision at the LLOQ was 16.3%; therefore, the accuracy and precision of the proposed method were all in

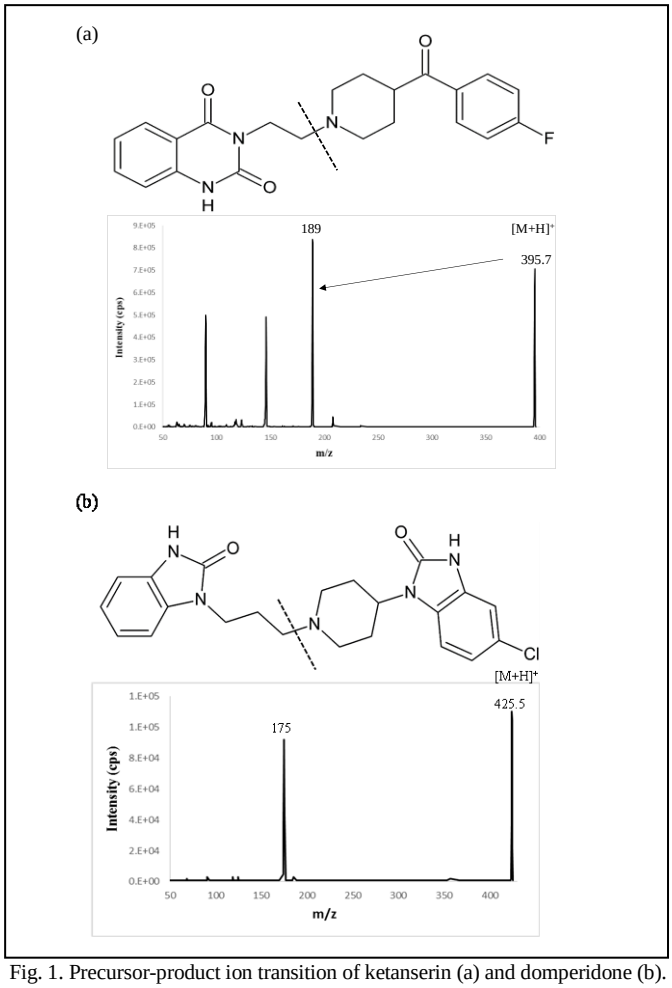

Fig. 1. Precursor-product ion transition of ketanserin (a) and domperidone (b).

accordance with Food and Drug Administration (FDA) regulations for the validation of bioanalytical methods [7]. The LLOQ was set at 1 ng/mL for ketanserin in 45 µL of drug- free plasma and the corresponding chromatograms of blank plasma and LLOQ are shown in Figure 2.

Short-term, long-term, freeze-thaw, post-preparative and stock stability storage conditions, as well as low QC stability (% recovery) and high QC stability (% recovery), are presented in Table 2. The mean integrated peak areas of the low QC (10 ng/mL) and high QC (5000 ng/mL) samples were compared before and after stability testing.

Matrix effects (n = 5) and recoveries (n = 5) of ketanserin in guinea pig plasma were 105.2 ± 6.42% and 98.5 ± 1.79%, respectively. Neither matrix effects nor percent loss exceeded 20%. The combined effects of all matrix components other than the target analyte were trivial; therefore, the current method had significant extraction efficiency. Overall, this method is not only faster and simpler than previous methods to analyze ketanserin in plasma, but it is also fully validated.

Table 1. The intra-, inter-day precision and accuracy of ketanserin (n=5)

Ketanserin	Nominal Concentration (ng/mL)	Mean Calculated Concentration (ng/mL)	CV%	RE%
Intra-day	1	1.05	16.33	5.09
	10	11.32	6.57	13.16
	1,000	1068.15	5.57	6.82
	5,000	4358.64	3.56	-12.83
Inter-day	1	0.91	11.69	-9.14
	10	11.07	7.61	10.66
	1,000	1071.55	3.17	7.16
	5,000	4344.04	5.48	-13.12

Table 2. Conditions and results of stability test of ketanserin in guinea pig plasma

Stability test	Storage condition	Low QC stability (%)	High QC stability (%)
Short term in plasma	*Room temperature for 6h*	97.31	105.22
Long term in plasma	*-70 ℃ for 14 days*	90.86	93.78
Freeze-thaw cycle in plasma	*-70 ℃ after the third cycle*	110.05	94.39
Process (extracted sample)	*4 ℃ for 24h*	108.40	93.37
Stock solution	*-20℃ for 14 days*	103.72	99.78

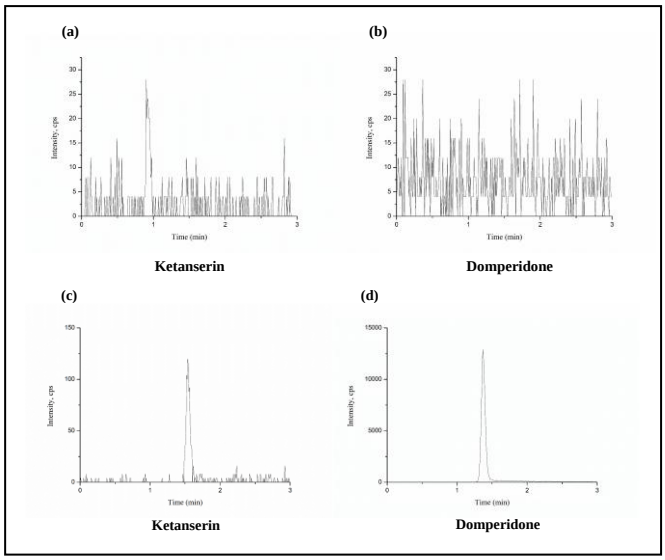

Fig 2. Chromatogram of blank plasma sample and LLOQ of ketanserin (a), (c) and domperidone (b), (d) in guinea pig analyzed by HPLC-MS/MS.

C. Application of the proposed method to a pharmacokinetic study

As a proof-of-principle, the present method was used to successfully analyze the pharmacokinetic parameters of ketanserin. The resulting pharmacokinetic graph of log-plasma concentration of ketanserin in guinea pig plasma after intravenous administration of 3 mg/kg ketanserin is presented in Figure 3. Results from the noncompartmental analysis, the maximum plasma concentrations of ketanserin (Cmax), half-life (t1/2), and the area under the plasma concentration versus time curve from 0 h to the last measurable concentration (AUCt) are given in Table 3.

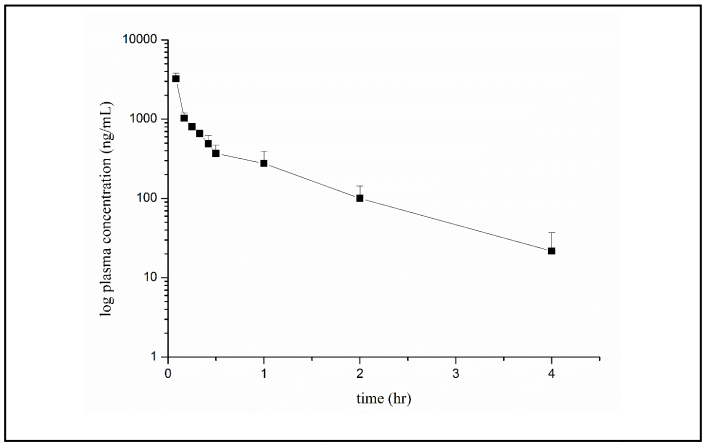

Fig 3. Log plasma concentration of ketanserin in guinea pig plasma after intravenous administration of 3mg/kg ketanserin

Table 3. Noncompartmental analysis result of ketanserin in guinea pig after intravenous administration of ketanserin 3 mg/kg.

	Ketanserin
C_{max} (ng/mL)	3224.0 ± 593.07
$t_{1/2}$ (hr)	0.85 ± 0.30
AUC_{last} (ng*hr/mL)	997.97 ± 245.14

IV. CONCLUSION

A novel LC-MS/MS MRM method was successfully developed to simultaneously determine the concentrations of ketanserin in plasma after intravenous administration of ketanserin in guinea pigs. Specificity, accuracy, precision, percent recovery, matrix effects, and stability of this method were all within the acceptable range of the FDA regulations for the validation of bioanalytical methods [7]. Furthermore, this simple, rapid, and accurate method was successfully applied to an in vivo pharmacokinetic study and yielded sufficient information on pharmacokinetic parameters. This method, therefore, is a better prospect to quantify ketanserin than currently available methods.

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