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Short communication

Liquid chromatography tandem mass spectrometry assay to determine the pharmacokinetics of aildenafil in human plasma

Jiang Wang [a], Yao Jiang [a], Yingwu Wang [a], Xia Zhao [b], Yimin Cui [b,**], Jingkai Gu [a,*]

[a] *Research Center for Drug Metabolism, College of Life Science, Jilin University, Changchun 130023, China*
[b] *Department of Pharmacy, Peking University First Hospital, Beijing 100034, China*

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Abstract

A simple, sensitive and specific liquid chromatography/tandem mass spectrometry method for the quantitation of aildenafil, a new phosphodiesterase V inhibitor, in human plasma is presented. The analyte and internal standard, sildenafil, were extracted by a one-step liquid–liquid extraction in alkaline conditions and separated on a C_{18} colunm using ammonia:10mM ammonium acetate buffer:methanol (0.1:15:85, v/v/v) as the mobile phase. The detection by an API 4000 triple quadrupole mass spectrometer in multiple-reaction monitoring mode was completed within 2.5 min. The calibration curve exhibited a linear dynamic range of 0.05–100 ng/ml with a 10 pg/ml limit of detection. The intra- and inter-day precisions measured as relative standard deviation were within 8.04% and 5.72%, respectively. This method has been used in a pharmacokinetic study of aildenafil in healthy male volunteers each given an oral administration of one of the three dosages.

Keywords: Aildenafil; LC–MS/MS; Pharmacokinetics

1. Introduction

Aildenafil, 1-{[3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1Hpyrazolo [4,3-*d*] primidin-5-yl)-4-ethoxyphenyl] sulfonyl}-*cis*-3, 5-dimethylpiperazine, has recently been developed by Beijing Board Long-live Spring High-tech Biopharm Company Ltd. (Beijing, PR China), and is currently being evaluated in phase III clinical trial. As a pyrrolopyrimidinone analogue of sildenafil and vardenafil, aildenafil is a potent and selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type V and the predominant isozyme metabolizing cGMP in the corpus cavernosum [1]. Its efficacy has been demonstrated in clinical trials by comparison with placebo and sildenafil, and the result shows that aildenafil has a longer duration of action than sildenafil in the treatment of male erectile dysfunction. Thus, aildenafil is more suitable for a low-dose, once-daily administration. Consequently, an appropriate method to measure the low concentration of aildenafil in human plasma was desired.

So far, various methods have been developed for the quantification of the sildenafil and vardenafil in biological samples, such as micellar electrokinetic capillary chromatography (MEKC) [2,3], gas chromatography–mass spectrometry (GC/MS) [4], electrochemical detection [5,6] and high-performance liquid chromatography (HPLC) with ultraviolet [7–11], Diode array [12], MS [13–15], and MS/MS [16–18] detection. However, to our knowledge, no reference to any assay method for aildenafil quantification could be found in literature. Moreover, the aforementioned methods [2–18] suffer from various limitations, such as low sensitivity, relatively complex sample preparation, and long analytical run time. Based on the sensitivity, selectivity, and high-throughput of LC–MS/MS methods, we report here the first quantitative method to determine aildenafil in human plasma with LC–MS/MS using sildenafil as the internal standard (I.S.). The method exhibited excellent performance in terms of selectivity, robustness, efficiency with a run time of 2.5 min per sample, and simplicity of liquid–liquid extraction sample preparation. Furthermore, it was fully validated and successfully applied to a pharmacokinetic study in healthy male volunteers treated with oral administration

* Corresponding author. Tel.: +86 431 5619955; fax: +86 431 5619955.
** Corresponding author. Tel.: +86 10 66110987.
E-mail addresses: cuiymzy@126.com (Y. Cui), gujk@mail.jlu.edu.cn (J. Gu).

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of 30 mg, 60 mg, 90 mg aildenafil (10 volunteers for each dosage).

2. Experimental

2.1. Chemicals and reagents

Aildenafil citrate and sildenafil citrate (purity >99.5% in each case) were kindly supplied by Beijing Board Long-live Spring High-tech Biopharm Company Ltd. (Beijing, PR China). Methanol was HPLC-grade, while all other chemicals were analytical grade and was used without further purification. Distilled water, prepared from demineralized water, was used throughout the study. Blank human plasma (drug free) was obtained from Changchun Blood Donor Service (Changchun, PR China).

2.2. Preparation of stock solutions

All concentrations of aildenafil and sildenafil refer to the free base. Stock solutions (100 μg/ml) of aildenafil citrate and sildenafil citrate were prepared separately in methanol. A series of aildenafil standard solutions with concentrations of 0.25, 0.50, 1.00, 3.00, 10.0, 30.0, 100 and 500 ng/ml were prepared by dilutions of aliquots of the stock solution with 50% methanol. A working I.S. solution (sildenafil, 100 ng/ml) and QC solutions (0.50, 10.0 and 400 ng/ml, respectively) were prepared in a similar way, stored at 4 °C and used within 1 month of preparation.

2.3. LC–MS/MS conditions

An Agilent 1100 series (Agilent Technologies, Palo Alto, CA, USA) binary pump and autosampler were used for solvent and sample delivery. A switching valve was used to prevent introducing the pre-eluent from entering the ion source. The chromatographic separation was performed on a Nucleosil C_{18} column (5 μm, 50 mm × 4.6 mm I.D. from Dalian Johnsson Separation Science and Technology Corp., Dalian, PR China) at 30 °C. The mobile phases consisted of ammonia:10 mM ammonium acetate buffer:methanol (0.1:15:85, v/v/v) pumped at a flow rate of 0.7 ml/min.

An Applied Biosystems Sciex API 4000 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) equipped with an electrospray ionization (ESI) source was used for mass analysis and detection. ESI was performed in the positive ion mode with nitrogen as the nebulizer, heater and curtain gas. The detector was operated at unit resolution in MRM mode using the transitions from the protonated molecular ions to product ions at m/z 489.4 → 113.2 and 489.4 → 99.1 for aildenafil, m/z 475.4 → 100.2 for sildenafil. A mixed solution of aildenafil and I.S. was infused through a syringe pump (10 μl/min) into the stream of mobile phase eluting from the column for tuning the instrument response and optimizing the high-level gas flow parameters, ionspray needle voltage, heater gas temperature, declustering potential (DP), and collision energies (CE) in the MRM mode. Optimum values for nebulizer, heater, and curtain gas flow rates were 60, 50 and 15 units (arbitrary), respectively. The ionspray needle voltage and heater gas temperature were set at 5200 V and 540 °C, respectively. The collision gas (N_2) was set at 5 units (arbitrary), the DP was chosen at 100 V, and CE of 44 eV (m/z 489.4 → 113.2) and 56 eV (m/z 489.4 → 99.1) were used for aildenafil, 45 eV was used for sildenafil (m/z 475.4 → 100.2), respectively. The instrument was interfaced to a computer running Applied Biosystems Analyst version 1.3.2 software.

2.4. Sample preparation

To 500 μl aliquot of human plasma were added 100 μl I.S. solution, 100 μl 50% methanol (or a standard or QC solution of aildenafil), and 100 μl 1M NaOH. The mixture was vortexed for 30 s and extracted by 2.5 ml ether-dichloromethane (60:40, v/v). After being shaken for 10 min and centrifuged at 3500 × g for 5 min, the organic phase was transferred to another tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 200 μl mobile phase and 40 μl was injected into the LC–MS/MS system. The samples with concentrations greater than the maximum standard in the calibration curve were quantified by dilution of these samples with blank plasma.

2.5. Method validation

Three independent calibration curves based on eight spiked plasma samples (0.05–100 ng/ml) on each of three separate days were prepared to validate the linearity of the method. Calibration curves were analyzed by weighted linear regression ($1/x^2$) of analyte-I.S. peak area ratios. Accuracy [as relative error (R.E.)] and intra- and inter-day precisions [as relative standard deviation (R.S.D.)] were assessed by assays of six replicates of LLOQ samples (0.05 ng/ml) and QC samples (0.1, 2.0 and 80.0 ng/ml) on three different days. The lower limit of quantitation (LLOQ) was the concentration below which the inter-day R.S.D. >20%. The limit of detection (LOD) was the concentration with signal-to-noise ratio of 3. Recoveries of aildenafil and I.S. were determined by comparing peak areas of extracted QC samples with those of post-extraction blank plasma spiked at corresponding concentrations.

Matrix effects for aildenafil and the I.S. were evaluated by comparing the peak areas of post-extraction blank plasma samples spiked with QC solutions with the areas obtained by spiking aqueous blanks with the corresponding standard solutions. Stability tests were evaluated using QC samples stored for 1 month at −20 °C and subjected to three freeze-thaw cycles. Stability of analyte and I.S. in the stock solution at 4 °C for a month and in mobile phase on storage in plastic autosampler vials under autosampler conditions for 12 h was also assessed.

Three concentrations (2000, 1000 and 500 ng/ml in plasma) were prepared and diluted to 100 ng/ml by blank plasma. The dilution effect of samples (0–20 times dilution in range of 100–2000 ng/ml) was evaluated by the diluted samples (six replicates each concentrations).

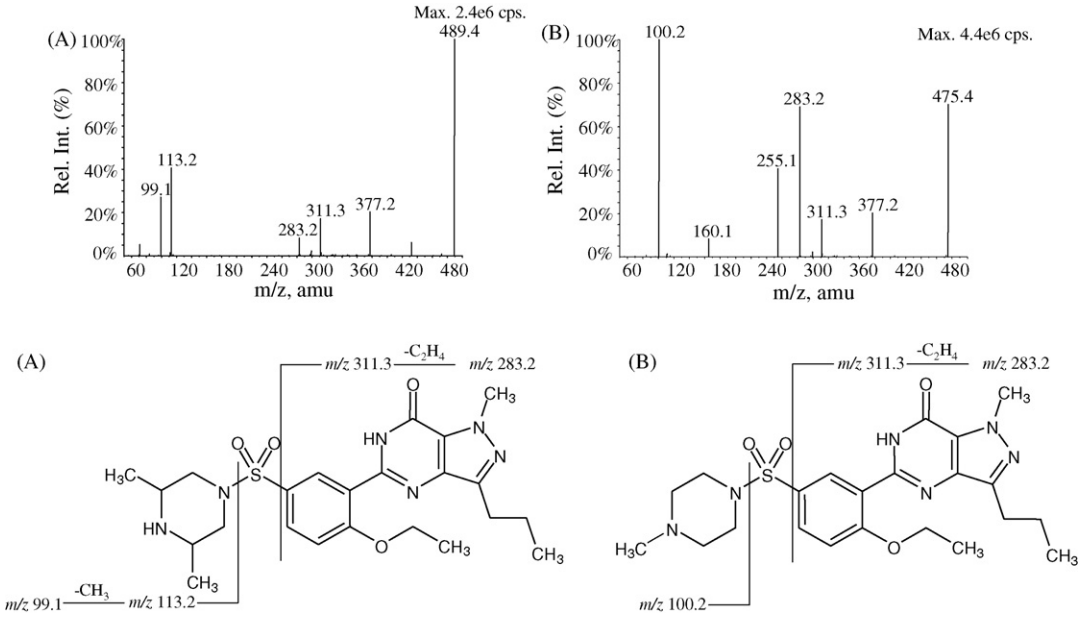

Fig. 1. Full-scan product ion spectra of $[M+H]^+$ and fragmentation pathways for (A) aildenafil and (B) sildenafil.

2.6. Pharmacokinetic study

Thirty healthy male volunteers participated in this study. None of them was alcohol abuser or was taking any concomitant medication during the study. They all read the protocol and gave written informed consent before entering the study.

After a 12 h fast, volunteers received a single tablet containing aildenafil (30 mg, 60 mg, and 90 mg ten persons for each dosage). Blood samples (1 ml) were collected prior to dosage and at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24, and 36 h thereafter and withdrawn into heparinized tubes immediately. After centrifugation of whole blood at 3000 × g for 10 min, plasma samples were obtained and stored at −20 °C. Pharmacokinetic parameters were calculated using Topfit 2.0.

3. Results and discussion

3.1. Mass spectrometry

Both analyte and I.S. responded best to the positive ionization mode, with the protonated molecular ions $[M+H]^+$ as the major species. Product ion spectra and fragmentation pathways of aildenail and sildenafil were shown in Fig. 1. The MRM acquisitions were performed at unit resolution using the transition *m/z* 489.4 → 113.2 and *m/z* 489.4 → 99.1 for aildenafil, *m/z* 475.4 → 100.2 for sildenafil, respectively. The transition *m/z* 489.4 → 113.2 was used for quantification of aildenafil because of its best response, while *m/z* 489.4 → 99.1 was used as the qualifier. The mass parameters were optimized by observing the maximum response obtained for the product ions.

3.2. Chromatography

A short Nucleosil C_{18} column was chosen from a number of the commercial columns evaluated (Nucleosil, Hypersil and Zorbax), because it gave the best chromatography with minimal matrix effects and a minimum run time. A better response was achieved by using methanol in place of acetonitrile as the organic component of the mobile phase. The inclusion of 10 mM ammonium acetate buffer reduced matrix effects and peak tailing without decreasing response, while the adjustment of ammonia improved the peak shape. The similar retention times of 1.42 and 1.44 min for I.S. and analyte, respectively, reduced potential matrix effects. This method required 500 μl of plasma and allowed high sample throughput (150–200 samples per day) due to a simple sample preparation procedure and short run time of 2.5 min. Otherwise, both aildenail and sildenafil gave a relatively high recovery on the solvent extraction.

Table 1
Precision and accuracy for the determination of aildenafil in human plasma (data are based on analysis of LLOQ and QC samples ($n=6$) on three different days)

Nominal conc. (ng/ml)	Calculated conc. (ng/ml)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)	Relative error (%)
0.05	0.05	6.99	2.07	0.87
0.10	0.10	8.04	4.52	4.03
2.00	2.04	3.81	5.72	1.92
80.0	84.8	6.48	2.13	6.01

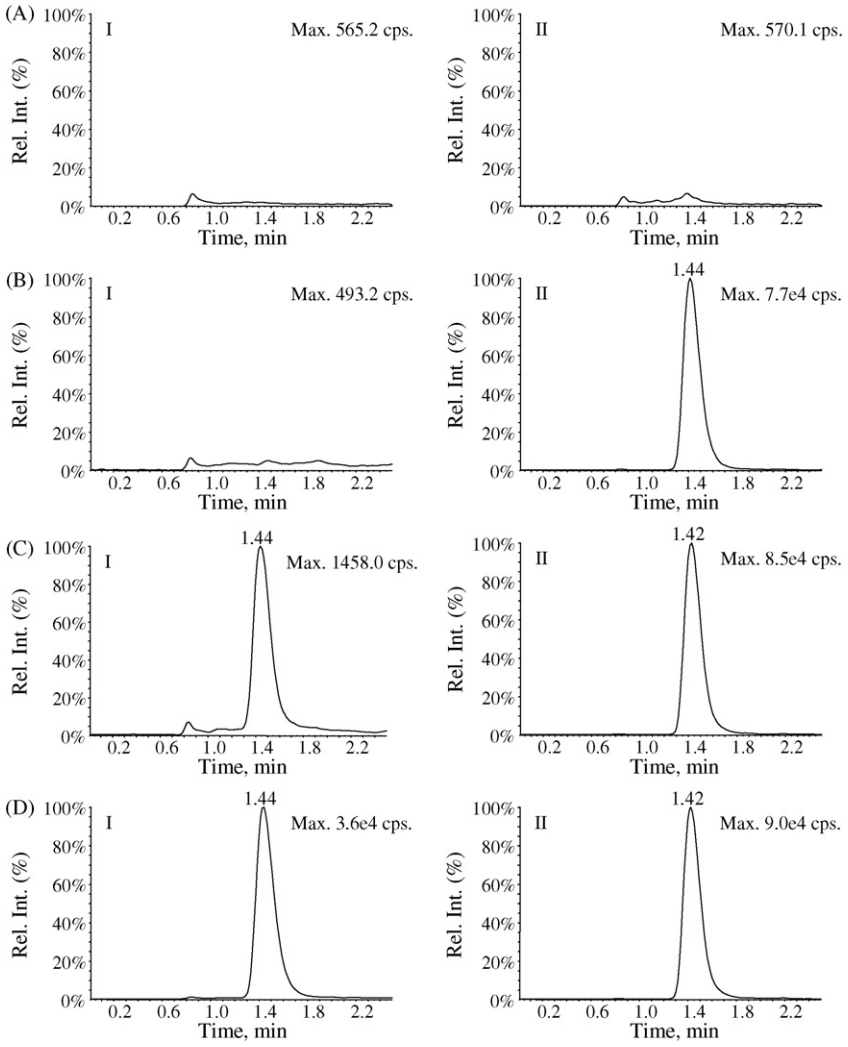

Fig. 2. Representative MRM chromatograms of (A) blank plasma; (B) blank plasma spiked with internal standard; (C) blank plasma spiked with aildenafil at the lower limit of quantitation (0.05 ng/ml); and (D) a plasma sample 12 h after administration of an oral dosage at 30 mg to a healthy male volunteer. Peak I, aildenafil; Peak II, sildenafil.

3.3. Assay validation

Typical chromatograms (the transition m/z 489.4 → 113.2 used for quantification) of blank plasma, blank plasma spiked with I.S., blank plasma spiked with aildenafil at LLOQ (0.05 ng/ml) and a study sample containing a low concentration of sildenafil are shown in Fig. 2. Linearity was achieved over the range 0.05–100 ng/ml ($r > 0.995$) with an LOD of 10 pg/ml. Precision and accuracy were satisfactory at the three concentrations studied (Table 1).

Recoveries of aildenafil at concentrations of 0.1, 2.0 and 80 ng/ml were 86.2%, 81.7%, and 79.6%, respectively. Matrix effects for aildfenafil and I.S. were minimal based on concentrations being 96.8–105.4% of nominal concentrations. Aildenafil was stable under all the storage conditions evaluated with mean recoveries of 94.3–101.6% of the nominal concentrations. The dilution effect for aildfenafil was 98.1–104.1% of nominal concentrations in the 0–20 times dilution.

3.4. Pharmacokinetic study

The mean plasma concentration versus time curves after administration of three dosages are shown in Fig. 3. The main pharmacokinetic parameters of aildenafil are listed in Table 2. After oral administration, aildenafil was absorbed rapidly and

Table 2
Pharmacokineticparameters of aildenafil

Parameter	Dosage of aildenafil		
	30 mg	60 mg	90 mg
C_{max} (ng/ml)	262.33 ± 157.89	688.90 ± 282.28	1388.6 ± 513.17
T_{max} (h)	1.44 ± 0.51	1.53 ± 0.57	1.50 ± 0.41
$T_{1/2}$ (h)	4.40 ± 0.36	3.97 ± 0.32	4.47 ± 0.74
$AUC_{0-\infty}$ (ng h/ml)	1428.6 ± 1032.9	4248.5 ± 1265.4	10042 ± 4507.6
Ke	0.16 ± 0.01	0.18 ± 0.01	0.16 ± 0.03

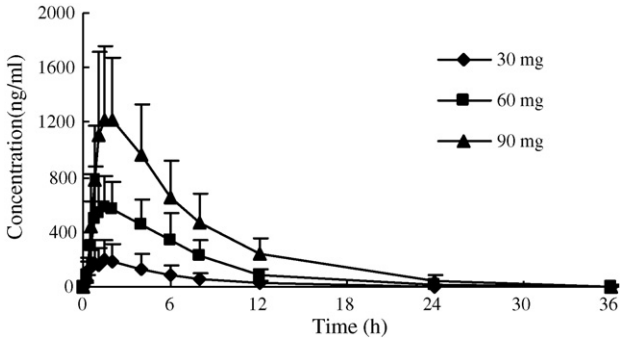

Fig. 3. Mean plasma concentration-time profile of aildenafil after administration of three dosages at 30 mg, 60 mg and 90 mg (ten volunteers each dosage). Data are mean ± S.D.

the drug was detected in plasma from the first blood sampling (0.25 h) and rapidly reached a maximum at T_{max} values: 1.44 ± 0.51, 1.53 ± 0.57, and 1.50 ± 0.41 h for dosages of 30, 60 and 90 mg, respectively. The elimination half-life of aildenafil (4.40 ± 0.36, 3.97 ± 0.32 and 4.47 ± 0.74 h) was similar for the three dose levels. There was a significant increase in C_{max} and $AUC_{0-\infty}$ with increasing dosages, suggesting that aildenafil may have linear pharmacokinetic characteristics in humans within the dose ranges tested. The high sensitivity of the present method allowed the measurement of trace levels of aildenafil in plasma (<0.1 ng/ml) 36 h after the low dose administration.

4. Conclusion

A highly selective, sensitive and rapid LC–MS/MS method was reported for the determination of aildenafil in human plasma. The sensitivity was sufficient to determine the drug level in human plasma after oral administration. The method allowed for high sample throughput, because of both the short run time and relatively simple sample preparation procedure.

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