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Determination of alprostadil in rat plasma by ultra performance liquid chromatography–electrospray ionization–tandem mass spectrometry after intravenous administration

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ABSTRACT

A rapid, highly selective ultra performance liquid chromatography–electrospray ionisation–tandem mass spectrometry method (UPLC–ESI–MS/MS) was developed and validated for the determination and pharmacokinetic investigation of alprostadil in rat plasma. After a simple sample preparation procedure involving a one-step liquid–liquid extraction, alprostadil and the internal standard, diphenhydramine, were chromatographed on an ACQUITY UPLC™ BEH C_{18} column with gradient elution using a mobile phase consisting of acetonitrile and water (containing 0.1% formic acid) at a flow rate of 0.25 mL min^{-1}. The detection was performed on a triple quadrupole tandem mass spectrometer in multiple reaction monitoring (MRM) mode via an electrospray ionization (ESI) source. The calibration curve was linear ($r^2 = 0.99$) over the concentration range 0.4–250.0 ng mL^{-1}, with a lower limit of quantification of 0.4 ng mL^{-1} for alprostadil. The inter- and intra-day precision (%R.S.D.) was less than 8.5% and 2.4%, respectively, and the accuracy (RE%) was between 9.3% and 1.0% ($n = 6$). Alprostadil in rat plasma was stable when stored at room temperature for 0.5 h and at −20 °C for two weeks. The method was very rapid, simple and reliable, and was employed for the first time for the pharmacokinetic studies of alprostadil in rats after a single intravenous administration of 50 μg kg^{-1}.

1. Introduction

Alprostadil, a hormone drug, has been used for a long time for the treatment of peripheral arterial occlusive disease [1–4] since it is able to effectively inhibit platelet aggregation. However, it is rapidly metabolized by the lungs and as much as 80% of a single dose may be metabolized in a single pass through the lungs [5,6]. Therefore, the alprostadil plasma concentrations are very low. Due to the difficulties in measuring alprostadil in plasma, there is very little pharmacokinetic data for alprostadil obtained under in vivo conditions.

Because of the clinical and biochemical significance of alprostadil, a great deal of effort has been devoted for developing suitable analytical methods for the determination of alprostadil in biological samples. The commonly used methods included gas chromatographic–tandem mass spectrometric (GC–MS/MS) and radioimmunoassay (RIA) [7,8]. Schweer et al. [7] developed a GC–MS/MS method for the determination of alprostadil in plasma and, although their method had a lower limit of detection (LOD) in the picogram order, it was time-consuming and complicated because of the multi-step derivatization procedure. Only eight samples could be analyzed per day according to this method. Hence, the GC–MS/MS method was not feasible for high-throughout determinations of biological samples. According to another report [8], the RIA method possessed a lower LOD for alprostadil of about 3.9 pg mL^{-1}. Nevertheless, this assay could produce radioactive contamination and required very specialized and expensive equipment. These problems restricted the further application of the RIA method. Up to now, several LC/MS methods have been investigated for the determination of alprostadil and an LC/MS method has been reported for the determination of alprostadil in human semen [9]. However, the lower limit of quantification (LLOQ) was higher than 5.0 ng mL^{-1}. Accordingly, it was not sensitive enough to determine alprostadil in plasma for pharmacokinetic studies after intravenous administration. Another LC/MS method was investigated for the determination of prostaglandin-related substances in human semen and rat brain [10]. During the sample preparation procedure, alprostadil needed to be acetylated using acetic anhydride. Moreover, the study did not provide any recovery data to establish the efficiency of the method. Thus, it was not suitable for the determination and pharmacokinetic studies of alprostadil in plasma either. In addition, none of the above methods were validated. To sum up, this paper describes the development and validation of an ultra performance liquid

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chromatography–electrospray ionisation–tandem mass spectrometry (UPLC–ESI–MS/MS) method.

The purpose of this investigation was to develop a rapid, high selective UPLC–ESI–MS/MS method with an LLOQ of alprostadil of 0.4 ng mL^{-1} in rat plasma. The total run time was only 2.0 min for each sample. A comprehensive study of the stability of alprostadil was also performed during our investigation. To our knowledge, this is the first report of the development, validation and application of a UPLC–ESI–MS/MS method for the determination of alprostadil in rat plasma after a single intravenous administration of 50 μg kg^{-1} alprostadil.

2. Experimental

2.1. Materials and reagents

Alprostadil (Lot No. 140659-200401) was provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Diphenhydramine (internal standard, IS) was a kind gift from the Department of Analytical Chemistry of Shenyang Pharmaceutical University (Shenyang, China). The structures of alprostadil and IS are shown in Fig. 1. Acetonitrile and formic acid (HPLC grade) were purchased from Dikma Company (Richmond Hill, NY, USA). Purified water, used throughout the entire experiment, was obtained using a Barnstead EASYpure® II RF/UV ultrapure water system (Dubuque, IA, USA). The water was passed through a 0.22-μm membrane filter before use. All other chemicals were of analytical grade.

2.2. Instrumentation and conditions

2.2.1. Ultra performance liquid chromatography

Liquid chromatography was performed on an ACQUITY™ UPLC system (Waters Corp., Milford, MA, USA) with an autosampler maintained at 4 °C. The separation was carried out on an ACQUITY UPLC™ BEH C_{18} column (50 mm × 2.1 mm i.d., 1.7 μm; Waters Corp., Milford, MA, USA) and the column temperature was maintained at 35 °C. The analysis was carried out by gradient elution using acetonitrile (*A*) and water (*B*, containing 0.1% formic acid) as the mobile phase at a flow rate of 0.25 mL min^{-1}. The gradient conditions of the mobile phase were as follows: *A* was increased linearly from initially 30% to 60% during the first 0.6 min, and was held for 0.8 min. After 1.4 min, the composition was reset to the initial composition and 0.6-min re-equilibration time was allowed. The injection volume was 5 μL using the partial loop mode.

2.2.2. Mass spectrometry

A Waters ACQUITY™ TQD triple quadrupole tandem mass spectrometer (Waters Corp, Manchester, UK) with an electrospray ionization (ESI) interface was used for mass analysis. The ESI source was operated in negative ionization mode for alprostadil, and in positive ionization mode for the IS. The optimal ESI source parameters for alprostadil were as follows: capillary 2.0 kV, cone voltage 32 V, extractor 3.0 V and RF 0.1 V. The ESI source parameters for IS were as follows: capillary 3.9 kV, cone voltage 30 V, extractor 4.0 V and RF 0.2 V. The temperature of the source and desolvation was set at 100 and 400 °C, respectively. Nitrogen was used as the desolvation gas (550 L h^{-1}) and cone gas (50 L h^{-1}) for nebulization. For collision-induced dissociation (CID), argon was used as the collision gas at a pressure of approximately 2.91×10^{-3} mbar. The collision energy was 15 eV for both alprostadil and IS. Quantification was carried out using the multiple reaction monitoring (MRM) mode. The fragmentation transitions for MRM were m/z 353.1 → 317.1 amu for alprostadil, and m/z 256.1 → 166.9 amu for IS, with a scan time of 0.02 s per transition. All data collected in centroid mode were acquired using MassLynx™ NT4.1 software (Waters Corp., Milford, MA, USA). Post-acquisition quantitative analyses were carried out using a QuanLynx™ program (Waters Corp., Milford, MA, USA).

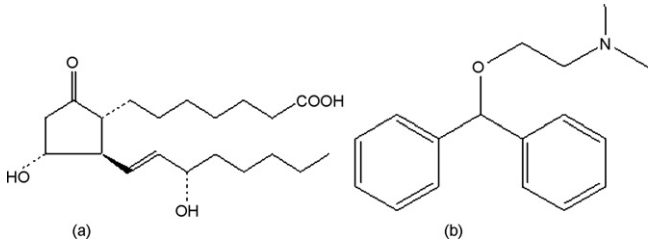

Fig. 1. The structures of alprostadil (a) and diphenhydramine (b).

2.3. Animals and blood sampling

The experimental protocol was approved by the University Ethics Committee for the use of experimental animals and all animal studies were carried out according to the Guide for Care and Use of Laboratory Animals.

Male Wistar rats, weighing 250 ± 20 g, were obtained from the Laboratory of the Animal Center of Shenyang Pharmaceutical University. Rats were housed in an environmentally controlled breeding room (temperature 22 ± 3 °C, relative humidity 45–60%) for one week before the experiments, and were fasted for 12 h but were allowed water ad libitum before drug administration. Then, 50 μg kg^{-1} alprostadil in physiological saline solution was administered to the rats via the femoral vein. Blood samples (0.3 mL) were collected from each rat by catheterization of the jugular vein at times of 0 (pre-dose), 0.33, 0.67, 1, 2, 4, 6, 10, 15, 30, 45 and 60 min, and transferred immediately to heparinized Eppendorf tubes (1.5 mL). The blood samples were centrifuged immediately at 4000 × *g* for 5 min at 2 ± 2 °C to obtain plasma samples.

2.4. Preparations of standard and quality control (QC) solutions

Alprostadil was dissolved in methanol to give a 1 μg mL^{-1} stock solution. The stock solution was then serially diluted with methanol to obtain working standard solutions of desired concentrations. IS working solution containing 4 ng mL^{-1} was prepared in the same manner. All working solutions were stored at −20 °C.

2.5. Preparation of calibration standards and quality control samples

The standard working solutions of alprostadil (20 μL) were used to spike blank rat plasma samples (100 μL), both in the pre-study validation and during the pharmacokinetic study. The calibration standards were prepared at concentrations of 0.4, 0.8, 2.0, 4.0, 10, 20, 50, 100 and 250 ng mL^{-1} in plasma. The QC samples used in the validation and the pharmacokinetic studies were prepared in the same way as the calibration standards at concentrations of 0.4, 0.8, 20 and 200 ng mL^{-1}, representing the LLOQ, low, medium and high concentrations, respectively.

2.6. Plasma sample preparation

All the plasma samples obtained after centrifugation were processed immediately. To a 100-μL aliquot of plasma sample were added 20 μL IS (4 ng mL^{-1} methanol solution) and 100 μL water. The mixture was then vortexed for 1 min and extracted with 3.0 mL ethyl acetate by shaking for 5 min in a test-tube shaker, then centrifuged at 4000 × *g* (2 ± 2 °C) for 10 min to separate the organic and aqueous phases. The supernatant organic phase was transferred to another polyethylene tube (5 mL) and evaporated to dryness

at 50 °C in a centrifugal concentrator (Labconco Corp., MO, USA). The residue was reconstituted in 50 μL methanol and vortex-mixed for 5 min. After centrifugation (2 ± 2 °C) at 16,000 × *g* for 10 min, the supernatant was transferred to an autosampler vial. Then, a 5-μL aliquot of the reconstituted extract was injected into the UPLC–ESI–MS/MS system for analysis.

2.7. Method validation

The validation of the bioanalytical method was performed in compliance with the FDA guideline [11]. Method validation usually includes selectivity, linearity, LLOQ, accuracy, precision, recovery, matrix effect and stability.

2.7.1. Selectivity

The selectivity of the method was investigated by analyzing blank plasma from six different rats, QC plasma samples and plasma samples after intravenous administration. Chromatograms were examined to determine the presence of any endogenous constituents which might potentially interfere with the analysis of alprostadil and IS.

2.7.2. Linearity and LLOQ

To assess linearity, plasma calibration curves (0.4–250.0 ng mL^{-1}) were prepared and assayed on 3 consecutive days. The calibration curves were fitted by linear least-square regression using $1/x^2$ as a weight factor of the peak-area ratios of alprostadil to IS versus alprostadil plasma concentrations. The peak-area ratios of alprostadil/IS of unknown samples or QC samples were then interpolated from the calibration curve to calculate the concentrations of alprostadil in the QC and test samples.

The LLOQ was defined as the lowest concentration on the calibration curve with acceptable precision and accuracy (six replicates with a relative standard deviation (R.S.D.) below 20% and relative error (RE) within ±20%).

2.7.3. Accuracy, precision and extraction recovery

The intra- and inter-run precision and accuracy were assessed by determining QC samples using six replicates at three concentrations on 3 different validation days. Precision was calculated as the R.S.D. within a single run and between different runs. Accuracy was expressed by RE, i.e. (determined concentration − nominal concentration)/(nominal concentration) × 100%. The intra- and inter-precision should not exceed 15% and the accuracy should be within ±15%, except for the low QC samples where the precision should be below 20% and the accuracy within ±20%.

The extraction recoveries of alprostadil were calculated by comparing the mean peak areas of alprostadil obtained from six extracted low, medium and high QC samples with those of six spike-after-extraction samples that represent 100% recovery. The extraction recovery of IS was estimated in the same manner in the medium QC samples.

2.7.4. Matrix effect

According to a published report [12], the quantitative measure of the matrix effect can be termed the matrix factor (MF). In our investigation, the IS normalized MF was determined and was defined as the ratio of the peak-area ratio of alprostadil/IS in the presence of matrix ions (*A*) to the peak-area ratio of alprostadil/IS in the absence of matrix ions (*B*), i.e. IS normalized MF = ($A/B \times 100\%$). Three concentrations of alprostadil in each of six replicates were studied. The value ($A/B \times 100\%$) was used to evaluate the matrix effect and, if it ranged from 85% to 115%, it was concluded that there was no significant matrix effect.

2.7.5. Stability

The stabilities of solutions kept at 4 °C for one week and at −20 °C for two weeks were estimated. Triplicate QC plasma samples with low and high concentrations were subjected to the conditions below. Storage stability was determined by assaying QC plasma samples after storage at −20 °C for two weeks. The freeze–thaw stability was investigated after three freeze (−20 °C)–thaw (room temperature) cycles. The short-term stability was assessed by analyzing QC plasma samples kept at ambient temperature for 0.5 and 1.0 h. The stability of alprostadil in the supernatant, which was determined by spiking the supernatant with QC solutions after extraction, was investigated at an ambient temperature for 2 h and the stability at 4 °C for 4 h was also studied. Post-preparative stability was assessed by analyzing the extracted QC plasma samples kept in autosampler vials at 4 °C for 4 h. Mean peak areas obtained from the analysis of the stored samples were compared to those obtained from the analysis of freshly prepared plasma samples.

3. Results and discussion

3.1. IS and extraction solvent

For LC–MS/MS quantification assay, a stable isotope-labeled analyte is the optimal IS. However, sometimes it was difficult to obtain such a reference standard. Moreover, an analog of the analyte could be an alternative IS, since it would exhibit similar behavior to the analyte during the entire sample extraction, chromatographic elution and mass spectrometric detection procedures. However, none of the analogs of alprostadil were stable, so they were not suitable for an IS. Finally, diphenhydramine was chosen as the IS, since diphenhydramine produces a stable ion with a high response and is eluted rapidly under the same UPLC–MS/MS conditions as alprostadil.

Currently, the most widely employed biological sample preparation method is liquid–liquid extraction (LLE). It was reported that the SPE can be employed for the clean-up of alprostadil [7,10], although there is no evidence to prove the method is efficient since no recovery data have been provided. Compared with protein precipitation (PPT), a one-step LLE could produce a relative clean sample and reduce the possibility of introducing highly polar materials into the column and MS system. Several organic extraction solvents were investigated, including ethyl acetate, ethyl acetate–diethyl ether (80:20, v/v), tert-butyl methyl ether (TBME), and *n*-hexane. The order of the extraction recovery for alprostadil was as follows, ethyl acetate > ethyl acetate–diethyl ether (80:20, v/v) > TBME > *n*-hexane. The ethyl acetate clearly produced the highest recovery for alprostadil. Ethyl acetate–diethyl ether (80:20, v/v) and TBME both exhibited a high extraction recovery for the IS, but both were less efficient for alprostadil (ca. less than 50%). *n*-Hexane produced a low recovery of alprostadil, as well as the IS. Therefore, ethyl acetate was chosen as the extraction solvent.

3.2. Mass spectrometry

The instrument parameters were adjusted to maximize the responses for alprostadil and IS by direct administration of their standard solutions into the mass spectrometer. Acquisition of mass spectrometry data for alprostadil standards was performed in negative ionization mode, while IS data were obtained in positive ionization mode. Alprostadil formed predominately deprotonated molecules at *m*/*z* 353.1 $[M\text{-}H]^-$ while the IS formed predominately protonated molecules at *m*/*z* 256.1 $[M+H]^+$ in the MS-scan mass spectra. The product ion spectra of alprostadil and IS are shown in Fig. 2. Alprostadil exhibited a higher fragment ion signal at *m*/*z* 317.1, formed by the elimination of two water molecules ([M-H-

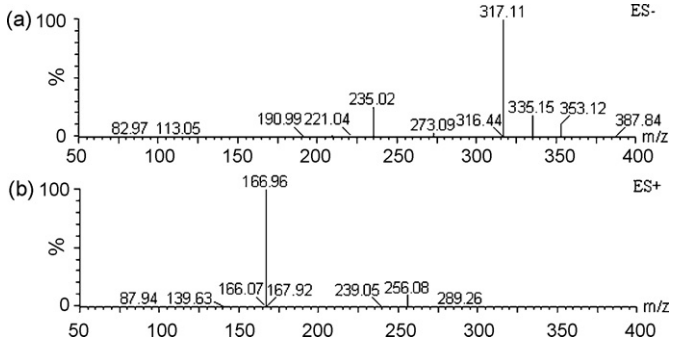

Fig. 2. Product ion spectra of alprostadil (a) and diphenhydramine (b).

36]$^-$), and this is confirmed in the literature [13]. Diphenhydramine exhibited a major fragment ion at m/z 166.9, produced from the loss of a neutral fragment of $[HOCH_2CH_2N(CH_3)_2]$.

Initially, acquisitions in positive mode for alprostadil were also tried, but no obvious $[M+H]^+$ ion signal was observed. Finally, two different ionization modes via ESI were employed to monitor alprostadil and IS. The mass transitions chosen for quantification were m/z 353.1 → m/z 317.1 for alprostadil, and m/z 256.1 → m/z 166.9 for IS.

3.3. Chromatography

The separation was performed on a column packed with sub-1.7 μm particles. The use of small particles of stationary phase allowed UPLC to extend the limits of both the peak capacity (due to higher efficiency) and speed of analysis (due to higher linear velocities) without compromising resolution [14]. Chromatographic separation was performed by gradient elution, since it markedly prolongs the column life, improves the chromatographic peak shapes, increases the response and shortens the elution time. The mobile phase was composed of acetonitrile–water (containing 0.1% formic acid) and the presence of a small amount of formic acid in the mobile phase assisted in reducing the retention time of the IS to 1.09 min, due to the alkaline nature of the IS.

Two other mobile phases, acetonitrile–water (containing 0.1% ammonium hydroxide) and acetonitrile–water, were also investigated. Alprostadil was eluted rapidly by acetonitrile–water (containing 0.1% ammonium hydroxide) within 0.6 min. The corresponding retention factors (k) approached 1.0 and, consequently, it was not suitable as the mobile phase. For the acetonitrile–water mixture, the signal-to-noise ratio of alprostadil was similar to that of alprostadil eluted with acetonitrile–water (containing 0.1% formic acid). However, the retention time of IS was prolonged and the peak shape of IS became broader and deteriorated. Consequently, acetonitrile–water (containing 0.1% formic acid) was used for the chromatographic separation.

A diverting valve, between the column and the mass spectrometer, was used to reduce contamination of the mass spectrometer. The UPLC flow passed into a waste container during the first 0.8 min of the elution process. It entered the mass spectrometer only when the analytes were eluted (0.8–2.0 min).

Two channels were used for recording. Channel 1 was for alprostadil with a typical retention time of 1.26 min, and channel 2 was for the IS with a typical retention time of 1.09 min. As shown in Fig. 3, both alprostadil and IS were well separated with excellent peak shapes. The very narrow chromatographic peaks with a peak width about 5 s, produced by UPLC™, resulted in an increase in the chromatographic efficiency and sensitivity. Both alprostadil and IS were rapidly eluted with retention times less than 2.0 min. The total run time was only 2.0 min per sample, which was much less than the literature values (more than 6 min) [10,13].

3.4. Method validation

3.4.1. Selectivity

Typical MRM chromatograms of alprostadil (peak I, channel 1) and IS (peak II, channel 2) in rat plasma samples are shown in Fig. 3, (a) is a blank plasma sample; (b) is a blank plasma sample spiked with alprostadil at the LLOQ of 0.4 ng mL^{-1} and IS (4 ng mL^{-1}); (c) is a plasma sample (with a plasma concentration of 208.2 ng mL^{-1} for alprostadil) from a rat at 20 s after a single intravenous administration of 50 μg kg^{-1} alprostadil. No interfering peaks were observed. The typical retention times for alprostadil and IS were 1.26 and 1.09 min, respectively.

3.4.2. Linearity and LLOQ

The peak-area ratios of alprostadil to the IS versus the nominal concentration displayed a good linear relationship over the concentration ranges of 0.4–250.0 ng mL^{-1} in rat plasma. Typical regression equations for 3 consecutive days are as follows:

the first day: $Y = 2.609 \times 10^{-3}\,x + 1.695 \times 10^{-3}$, $r = 0.9918$
the second day: $Y = 2.695 \times 10^{-3}\,x + 1.508 \times 10^{-3}$, $r = 0.9962$
the third day: $Y = 2.731 \times 10^{-3}\,x + 1.654 \times 10^{-3}$, $r = 0.9952$

where, Y represents the peak-area ratio of alprostadil to the IS and x represents the concentration of alprostadil in rat plasma. The precision of the slope of calibration curves above was 2.3%, and that of the intercept was 6.1%.

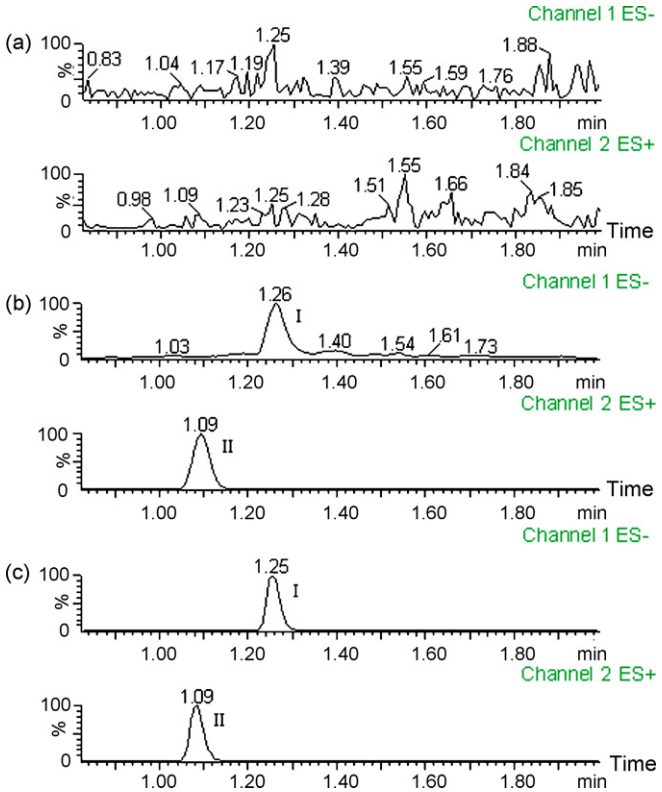

Fig. 3. Representative MRM chromatograms of alprostadil (peak I, channel 1) and IS (peak II, channel 2) in rat plasma samples: (a) blank plasma sample; (b) blank plasma sample spiked with alprostadil at the LLOQ of 0.4 ng mL^{-1} and IS (4 ng mL^{-1}); (c) plasma sample (with a plasma concentration of 208.2 ng mL^{-1} for alprostadil) from a rat at 20 s after a single intravenous administration of 50 μg kg^{-1} alprostadil.

Table 1
Accuracy and precision for the analysis of alprostadil in rat plasma (n = 3 days, six replicates per day).

Added concentration (ng mL^{-1})	Detected concentration (ng mL^{-1})	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)	RE (%)
0.4	0.45	14.1	–	11.5
0.8	0.71	13.6	10.7	−11.3
20.0	18.36	9.2	8.2	−8.2
200.0	210.41	8.0	7.1	5.2

The LLOQ for alprostadil was established at 0.4 ng mL^{-1}. The precision and accuracy at this concentration were acceptable, with an R.S.D. of 14.1% and an RE of ± 11.5%.

3.4.3. Precision, accuracy and extraction recovery

The method showed good precision and accuracy. The intra- and inter-day precision and accuracy of the assay were investigated by analyzing QC samples. All the values are summarized in Table 1. The intra-day R.S.D. was below 13.6% and the inter-day R.S.D. was below 10.7% while the RE (accuracy) was within ±11.3%. Hence, the method was proved to be both accurate and precise.

The clean-up of the plasma samples was achieved through a one-step LLE procedure. Ethyl acetate was applied as an extraction solvent and the recoveries of alprostadil at concentrations of 0.8, 20.0 and 200.0 ng mL^{-1} were 77.0 ± 2.0%, 74.6 ± 2.5% and 75.1 ± 1.0%, respectively. In addition, the recovery of the IS was 65.4 ± 3.5% and the recovery of alprostadil and the IS met the requirement for the analysis of biological samples.

3.4.4. Matrix effect

An investigation was carried out to evaluate the possibility of a matrix effect caused by ionization competition between the analytes and co-eluents when using LC–MS–MS for analysis. The IS normalized MFs were in the range of 93.3–104.5%. These results show that no endogenous species interfered with the ionization of the analyte.

3.4.5. Stability

The working solutions exhibited no obvious signs of degradation after one week of storage at 4 °C. For the solutions stored at −20 °C for two weeks, the same result was obtained. Alprostadil was shown to be stable (RE within ±11.8%) in rat plasma for two weeks when stored at −20 °C. Alprostadil was found to degrade by 15.8% after three freeze–thaw cycles in rat plasma. It has been reported that alprostadil degrades rapidly in rat plasma at 37 °C [15]. In our experiment, it was also observed that the unprocessed QC samples degraded by about 11.4% after 0.5 h at room temperature, and by about 23.1% after 1.0 h at room temperature. The analyte was stable (the deviation ranging from 87.3% to 109.2%) in the supernatant after extraction for 2 h at ambient temperature and stable (the deviation ranging from 90.3% to 110.2%) for 4 h at 4 °C. Alprostadil was found to be stable (RE within ±6.7%) in the reconstitution solution at 4 °C for 4 h. Taking all the above observations into consideration, the plasma samples should be extracted immediately after centrifugation.

3.5. Pharmacokinetic studies

The presented method was successfully applied to determine alprostadil in rat plasma obtained from six rats following a single intravenous administration of 50 μg kg^{-1} alprostadil. The profiles of the mean plasma concentrations versus time are shown in Fig. 4. The pharmacokinetic results were processed using drug and statistics (DAS) software, version 2.0 (Mathematical Pharmacology Professional Committee of China, Shanghai, China). The corresponding pharmacokinetic parameters of alprostadil are presented in Table 2. The plasma concentration of alprostadil fell rapidly after intravenous administration of alprostadil. The calculated elimination half-life ($t_{1/2\gamma}$) was 4.233 min, which was similar to the pharmacokinetics of alprostadil in humans described in the literature [16].

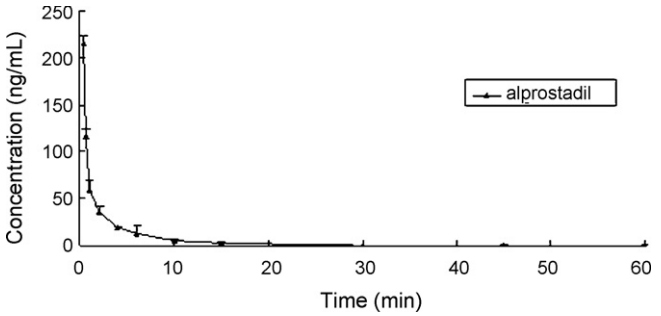

Fig. 4. Mean plasma concentration–time profile of alprostadil following a single intravenous administration of 50 μg kg^{-1} to six male rats.

Table 2
The main pharmacokinetic parameters of alprostadil after a single intravenous administration of 50 μg kg^{-1} alprostadil to six male rats.

Parameter	Unit	Value
[a] $t_{1/2\alpha}$	min	0.234 ± 0.292
[b] $t_{1/2\beta}$	min	0.239 ± 0.682
[c] $t_{1/2\gamma}$	min	4.23 ± 2.28
[d] V1	L kg^{-1}	0.098 ± 0.045
[e] CL	L min^{-1} kg^{-1}	0.092 ± 0.010
[f] AUC(0–t)	μg min L^{-1}	440 ± 76
[f] AUC(0–∞)	μg min L^{-1}	546 ± 64
[g] K10	min^{-1}	0.938 ± 0.388
[g] K12	min^{-1}	0.055 ± 0.386
[g] K21	min^{-1}	2.91 ± 1.44
[g] K31	min^{-1}	0.366 ± 0.188
[g] K13	min^{-1}	1.76 ± 0.80

[a] Rapid-distribution half-life.
[b] Slow-distribution half-life.
[c] Elimination half-life.
[d] Apparent volume of distribution.
[e] Clearance.
[f] Area under Concentration–time curve.
[g] Rate constant.

4. Conclusion

A UPLC–ESI–MS/MS method for the quantification of alprostadil in rat plasma was developed and validated. The method is rapid, and highly selective with an LLOQ at 0.4 ng mL^{-1} for alprostadil using 100 μL rat plasma. The total run time was only 2.0 min per sample and the plasma sample pretreatment was a one-step LLE procedure. The results obtained indicate that it is suitable for routine analysis of large batches of biological samples. It is superior to any other reported methods to evaluate the pharmacokinetics of alprostadil in rats after an intravenous administration of 50 μg kg^{-1} alprostadil. The simple preparation, speed of separation, and reproducibility of analysis are the most outstanding characteristics of this method.

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References

[1] M. Hirai, R. Nakayama, Br. J. Surg. 73 (1986) 20–23.
[2] H. Sinzinger, I. Virgolini, J. O'Grady, Prog. Clin. Biol. Res. 301 (1989) 85–96.
[3] J. Thum, L. Caspary, A. Creutzig, K. Alexander, VASA 27 (1998) 100–105.
[4] T. Weiss, H. Eckstein, C. Weiss, C. Diehm, Vasc. Med. 3 (1998) 171–175.
[5] J.W. Cox, N.A. Andreadis, R.C. Bone, R.J. Maunder, R.H. Pullen, J.J. Ursprung, M.J. Vassar, Am. Rev. Respir. Dis. 137 (1988) 5–12.
[6] M. Golub, P. Zia, M. Matsuno, R. Horton, J. Clin. Invest. 56 (1975) 1404–1410.
[7] H. Schweer, C.O. Meese, B. Watzer, H.W. Seyberth, Biol. Mass Spectrom. 23 (1994) 165–170.
[8] G. Hotter, J. Roselló-Catafau, D. Closa, G. Bioque, E. Gelpí, A. Javerbaum, E. González, M.A. Gimeno, J. Chromatogr. A 655 (1993) 85–88.
[9] J. Abian, E. Gelpi, J. Chromatogr. 394 (1987) 147–153.
[10] M. Yamane, A. Abe, J. Chromatogr. 568 (1991) 11–24.
[11] Guidance for Industry, Bioanalytical Method Validation, USFDA, http://www.fda.gov/cder/guidance/4252fnl.pdf, 2001.
[12] C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, Pharm. Res. 24 (2007) 1962–1973.
[13] Y. Xu, G. Xu, Chinese J. Chromatogr. 23 (2005) 633–635.
[14] Y. Ma, F. Qin, X. Sun, X. Lu, F. Li, J. Pharm. Biomed. Anal. 43 (2007) 1540–1545.
[15] H. Pan, P. Kopeckova, J. Liu, D. Wang, S.C. Miller, J. Kopecek, Pharm. Res. 24 (2007) 2270–2280.
[16] W. Cawello, A. Leozhardt, H. Schweer, H.W. Seyberth, R. Bonn, A.L. Lomeli, Br. J. Clin. Pharmacol. 40 (1995) 273–276.