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Determination of beraprost in human plasma by a high-performance liquid chromatography–tandem mass spectrometry

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Abstract

A simple, rapid, sensitive and specific liquid chromatography–tandem mass spectrometry method was developed and validated for quantification of beraprost, a stable, orally active prostacyclin analogue with vasodilatory, antiplatelet and cytoprotective effects. The analyte and internal standard, indomethacin, were extracted by solid-phase extraction using OASIS HLB cartridge. The chromatographic separation was performed on a C_{18} column with a mobile of 0.1% formic acid–methanol (30:70, v/v). The highest daughter ion of deprotonated analyte was quantitated in negative ionization by multiple reactions monitoring with a mass spectrometer. The mass transitions m/z 397 > 269 and m/z 356 > 312 were used to measure beraprost and internal standard, respectively. The assay exhibited a linear range from 0.02 to 2 ng/mL for beraprost in human plasma. The lower limit of quantitation was 20 pg/mL with a relative standard deviation of less than 20%. The method was validated with respect to linearity, sensitivity, specificity, recovery, accuracy and precision. The validated method has been successfully used to analyze human plasma samples for application in pharmacokinetic study.

Keywords: Beraprost; Prostacyclin; Tandem mass spectrometry; Pharmacokinetics

1. Introduction

Beraprost is a synthetic analogue of prostacyclin (Fig. 1, Mw = 398.5 g/mol) in which the unstable enol-ether has been replaced by a benzofuran ether function [1,2]. This modification increases the plasma half-life from 30 s to several hours, and permits the compound to be taken orally [3,4]. Doses of 20–100 μg in humans, given 1–3 times per day, have been demonstrated to improve clinical end points in diseases responsive to prostacyclin [5–8]. It has been studied for use in avoiding reperfusion injury [9–11]. Beraprost also inhibits platelet aggregation in healthy subjects and in diabetic patients at similar doses [4,12].

There have been few papers with regard to biotransformation and pharmacokinetics for beraprost. Although several investigations described some aspect of kinetic profile of beraprost, they did not lead to a better understanding of the pharmacokinetic characteristics [3,13]. Moreover, they focused on the pharmacodynamic effect of beraprost.

The practical assay for beraprost is important in evaluating the pharmacokinetic parameters. Interestingly, the analytical method published for beraprost has been very sparse even if it had been used for some time. This paper describes a fast, selective and highly sensitive approach, which enables the determination of beraprost in biological fluids with good accuracy using liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). The method was validated to assess its linearity, sensitivity, specificity, recovery, accuracy, precision and stability, and applied to the pharmacokinetic study in healthy volunteers after oral administration of beraprost tablets.

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2. Experimental

2.1. Chemicals

Beraprost reference standard as the sodium salt was obtained from Cayman Chemical Company (Ann Arbor, USA). Indomethacin as an internal standard (IS) and ammonium formate obtained from Sigma–Aldrich Co. (St. Louis, USA). Chemical structures are presented in Fig. 1. HPLC grade methanol and acetonitrile were purchased from Burdick & Jackson (Muskegon, USA). Analytical grade formic acid was purchased from Hayasi Pure Chemical Industries (Osaka, Japan).

2.2. LC–MS/MS conditions

LC–MS/MS analysis was performed on a Quattro Premier XE (Waters, Milford, USA) equipped with an electrospray ionization (ESI) source. The mobile phase was delivered using an Alliance 2795 HT HPLC system (Waters, Milford, USA). Chromatographic separations were performed on Zorbax RX C_{18} column, 5 μm particle size, 2.0 mm *L* × 150 mm *ID* (Agilent, Milford, USA) at 30 °C. The mobile phase composition was a mixture of 0.1% formic acid–acetonitrile (30:70, v/v). The flow rate was 0.3 mL/min and total run time was 3 min.

The Z-spray electrospray source was maintained at 120 °C. Nitrogen nebulization was performed with a nitrogen flow of 650 L/h. Argon was used as collision gas. The analytes were detected by multiple reaction monitoring (MRM) scan mode with negative ion detection, the parameter settings used were: capillary voltage at 2.7 kV, cone voltage at 38 V, collision cell entrance potential at 0.0 V, collision energy at 21 V, collision exit potential at 1.0 V, collision cell gas pressure at 3.00E−3 mbar, multiplier at 650 V and dwell time of 0.20 s.

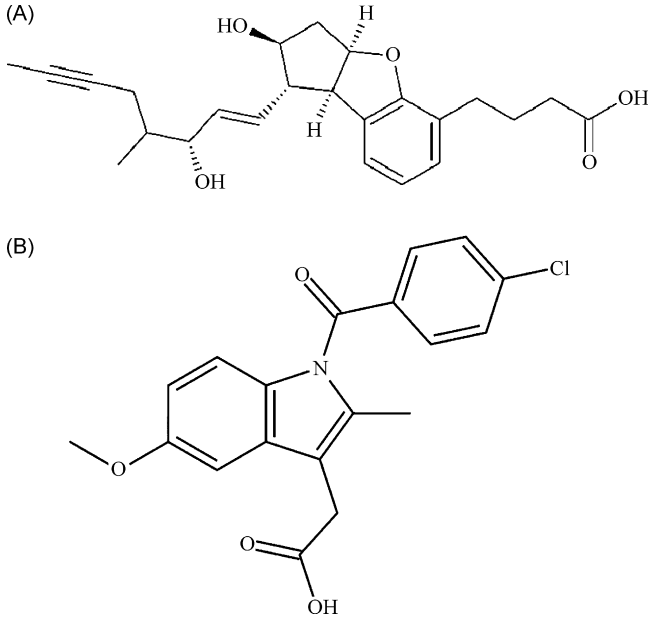

Fig. 1. Structure of beraprost (A) and indomethacin (B) as internal standard.

2.3. Stock and working solutions

The stock solutions of beraprost (100 μg/mL) and indomethacin (100 μg/mL) were prepared by dissolving appropriate amounts of the compounds in methanol–water (50:50, v/v) and stored at −20 °C. Working solutions were prepared by sequential dilution of the stock solution and stored at 4 °C. All solutions were stored in the dark and under refrigeration.

2.4. Sample processing

A 2 mL volume of plasma sample was transferred to a 15 mL polypropylene tube, and then 10 μL IS (1 μg/mL) was spiked and acidified with 1 mL 3% formic acid. After vortexing for 10 s, the sample was left for 10 min at room temperature for complete acidification.

Beraprost and IS were extracted from plasma samples using an OASIS HLB solid-phase extraction cartridge (3 mL/60 mg, Waters, Milford, USA). The cartridge with the highest recovery at 3 mL elution volume was conditioned with 3 mL methanol and 3 mL water and 3 mL 1% formic acid. The sample was slowly loaded on the cartridge using low vacuum at an approximate flow rate of 0.5 mL/min. The cartridge was washed with 3 mL 5% methanol in water containing 0.1% formic acid. The cartridge was dried at full vacuum for 5 min. The compounds were eluted with 3 mL methanol–10 mM ammonium formate (90:10, v/v). The eluate was evaporated to dryness under stream of nitrogen at 40 °C. The dry residue was reconstituted in 100 μL acetonitrile–water (70:30, v/v).

2.5. Validation

Aliquots of 200 μL working solutions were added to 1.8 mL blank plasma to obtain beraprost concentrations of 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2 ng/mL for calibration standard. Quality control (QC) samples were prepared in bulk, at concentration of 0.02, 0.1, 0.5, 1.5 ng/mL.

A calibration curve was constructed from a blank sample, a zero-blank sample with only IS and seven calibrators covering the total range (0.02–2 ng/mL), including the lower limit of quantitation (LLOQ, 0.02 ng/mL). Such calibration curves were generated on 5 consecutive days. Linearity was assessed by a weighed ($1/x$) least squares regression analysis. The acceptance criteria for each back-calculated standard deviation were 15% from the nominal value except for LLOQ, which was set at 20%.

The intra-day precision and accuracy were determined by analyzing five sets of QC samples in a day. The inter-day precision and accuracy were determined by analyzing five sets of QC samples on 5 different days. The acceptance criteria of intra- and inter-day precision were 20% or better for LLOQ and 15% or better for the rest concentrations and for accuracy were 100 ± 20% or better for LLOQ and 100 ± 15% or better for the rest concentrations.

Matrix effect on beraprost from the extraction procedure was determined by comparison of the peak area of beraprost in spiked plasma samples to the peak area of beraprost in extracted plasma samples prior to spiking with the same amounts of beraprost.

3. Results and discussion

3.1. Method development

We have developed a rapid and sensitive method for the determination of beraprost in human plasma by SPE and LC–MS/MS for pharmacokinetic study. The full scan first quadrupole negative ion spectrum and product ion spectrum of beraprost and IS are shown in Fig. 2. $[M-H]^-$ was deprotonated ion in the first quadrupole negative ion spectrum and was used as the precursor ion to obtain product ion spectra. The most sensitive mass transition was from *m/z* 397 to 269 for beraprost and from 356 to 312 for the IS. Multiple reactions monitoring (MRM) mode were used for quantitation and achieved very high sensitivity and selectivity.

Different mobile phase systems consisting of water–methanol (50:50, v/v) or water–acetonitrile (50:50, v/v), acetonitrile–0.1% formic acid (50:50, v/v) and acetonitrile–10 mM ammonium acetate (50:50, v/v, pH 3.5) were evaluated to improve separation and enhance sensitivity in MS. Buffers such as formic acid and ammonium acetate were added. The best signal was achieved using 0.1% formic acid–acetonitrile (30:70, v/v). The percent of formic acid was optimized to maintain good peak shape while being consistent with good ionization and fragmentation in the mass spectrometer.

The tandem mass spectrometer allows the selective detection of substances with varying masses or fragments without chromatographic separation. The development of the chromatographic system was focused on short retention times, paying attention to matrix effects as well as good peak shape. A high proportion of organic solvent, 0.1% formic acid–acetonitrile (30:70, v/v), was used to elute the analyte and the IS at retention times of 1.3 and 2.0 min, respectively. Flow rate of 0.3 mL/min produced a good peak shape and brought the run time to 2.80 min.

A stable isotope-labeled analyte has to be used as IS to deal with sample matrix effects. Since such an IS is not commercially available, an alternative approach has been used. The IS substance should match the chromatographic retention, recovery and ionization properties with matrix of beraprost. Indomethacin was found to theses criteria sufficiently. Hence indomethacin was chosen as the IS in the quantitative assay for beraprost from plasma.Validation

The calibration curve was linear over the concentration range 0.02–2 ng/mL for the analyte. The calibration curve gave acceptable results for the analyte and was used for all calculations. The calibration model was selected based on the analysis of the data by linear regression and weighing factors. The residuals were improved by weighed ($1/x$) least-squares linear regression. The best fit for the calibration curve could be achieved with the linear equation $y = mx + b$ with a $1/x$ weighing factor. The linear regression equation of a calibration curve for the analyte was $y = 0.84540x - 0.00236$ where y is the peak area ratio of analyte to the IS and x is the concentration of the analyte. The mean correlation coefficient of the weighed calibration curve generated during the validation was 0.999 for the analyte. Table 1 summarizes the calibration curve results for the analyte.

The selectivity of the method was examined by analyzing blank plasma extract and an extract spiked with only the IS.

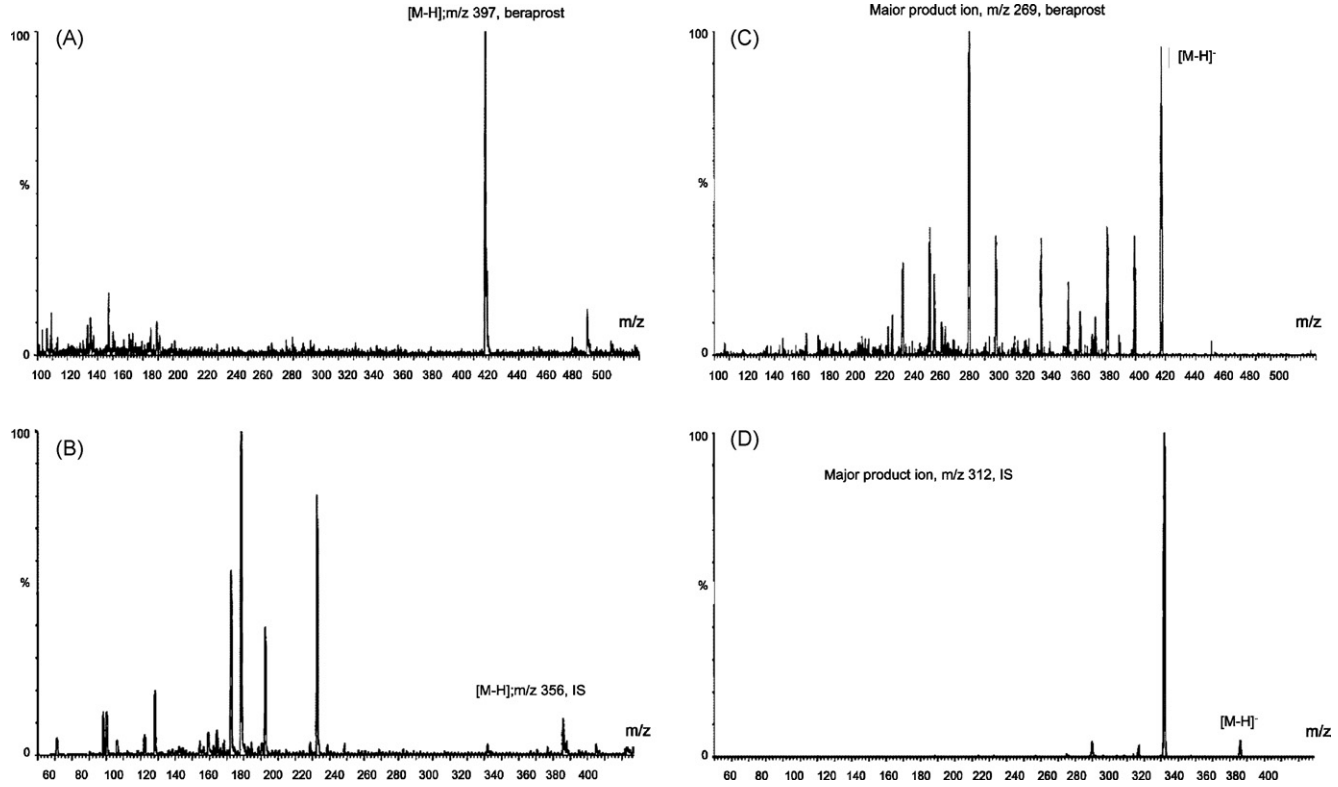

Fig. 2. Full scan first quadropole spectrum of (A) beraprost (*m/z* 397), (B) indomethacin (356) and product ion spectrum of (C) beraprost (*m/z* 397 > 269), and (D) indomethacin (*m/z* 356 > 312).

Table 1
Calibration curve of beraprost in human plasma for linearity and reproducibility ($n = 5$)

Replicate	Correlation coefficient (r^2)	Slope	Intercept
1	0.99875	0.83850	−0.00603
2	0.99935	0.87408	0.00515
3	0.99969	0.84278	−0.00301
4	0.99994	0.82875	−0.00105
5	0.99928	0.84334	−0.00685
Mean	0.99940	0.84540	−0.00236
S.D.		0.01706	
R.S.D.		2.0	

No significant interference in the blank plasma traces was seen from endogenous substances in drug-free plasma and chromatographic separation has been exhibited in Fig. 3.

Owing to the components of the sample matrix, ion suppression or enhancement may occur. These matrix effects in the LC–MS/MS method were evaluated by spiking the same concentration level of beraprost and the IS with five independent blank plasma lots. The MS/MS response and precision were then compared. If the responses and precision vary among each blank plasma lots, the matrix effect exists. Otherwise there was no significant difference for the peak response and precision. This effect was most likely due to the sample cleanup with SPE.

The extraction recovery of beraprost was 63.0% or 73.6%, respectively, on average (five replicates), and the dependence on concentration is negligible. The recovery of the analyte and the IS was low, but it was consistent, precision and reproducible. With the consistency in the recovery of beraprost and IS, the assay has proved to be robust in high-throughput bioanalysis.

The LLOQ over 10:1 in signal to noise ratio was 0.02 ng/mL with coefficient of variation of 6.4–13.4% and accuracy of 98.5–106.7%. The intra-day accuracy was 99.5–102.2% with precision of 4.7–5.5% and the inter-day accuracy was 98.1–100.5% with precision of 4.8–8.3% for 0.1, 0.5 and 1.5 ng/mL concentrations in human plasma. The results are presented in Table 2.

The stability of beraprost and the IS for stock solution (6 h at room temperature), short-term (24 h at room temperature), freeze-thaw (3 cycles), post-preparative (24 h in autosampler at 4 °C), and long-term (1 month at −70 °C) was evaluated in the dissolution solvent and in human plasma. It was found that beraprost and the IS were stable during each experiment (data not shown).

3.3. Application to biological samples

The validated method has been successfully used to determine concentration of beraprost in human plasma after the administration of 0.06 mg (0.02 mg × 3 tablets) oral dose of beraprost sodium. The mean concentration versus time profile of 12 subjects and plasma pharmacokinetic parameters (mean ± S.D.) after beraprost oral dosing were shown in Fig. 4. The serum concentration–time course of beraprost was fairly rapid with half-life averaged 0.408 ± 0.025 h. The maximum blood concentration was 0.420 ± 0.029 ng/mL. The area under the serum concentration–time curve (AUC) was estimated at 886 ± 267 pg/mL/min for up to 6 h (Fig. 4). The concentration of beraprost at 5 and 6 h was below the quantitative level, revealing that beraprost declined at a fast rate from the systemic circulation after oral dosing. Other investigation with multiple administration also reported rapid and linear elimination of beraprost with

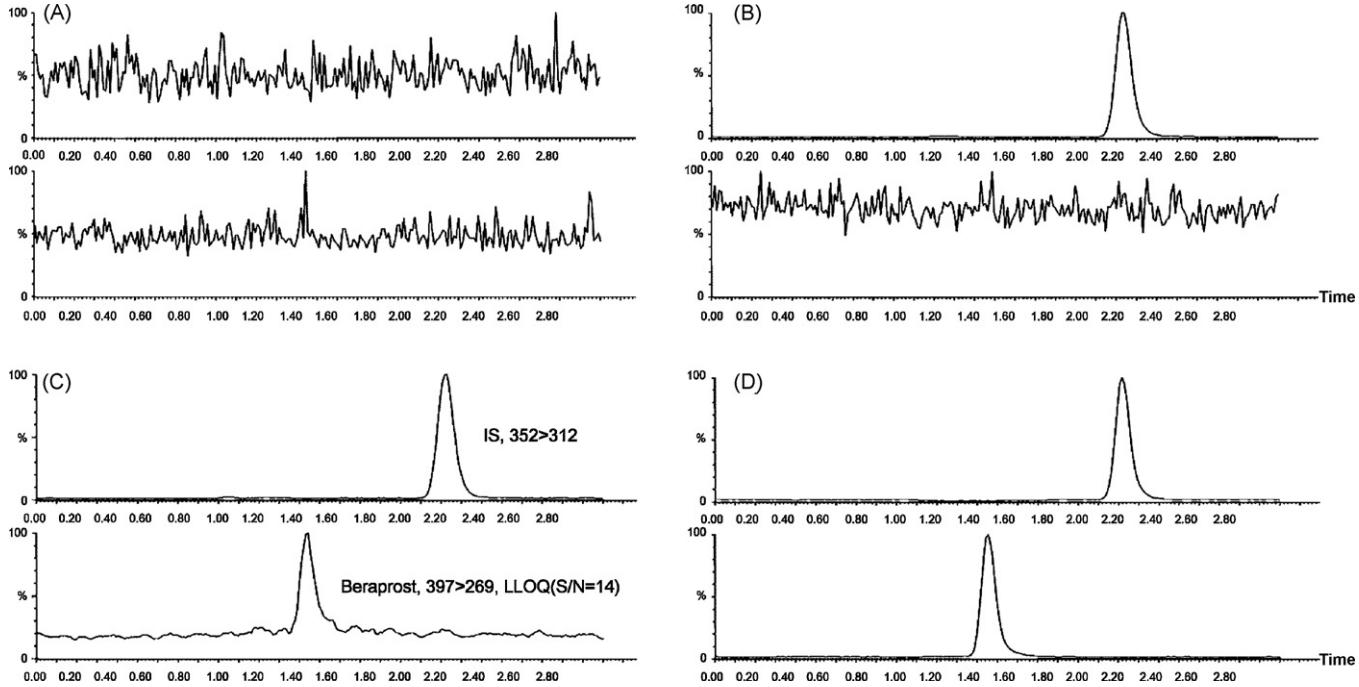

Fig. 3. LC–MS/MS chromatograms of (A) blank plasma, (B) zero-blank plasma with only IS, (C) plasma spiked with IS and an LLOQ sample (0.02 ng/mL), and (D) plasma (0.31 ng/mL) from a volunteer at 1.5 h after the administration of 0.06 mg (0.02 mg × 3 tablets) dose of beraprost.

Table 2
Intra- and inter-day precision and accuracy of measurement of beraprost in human plasma ($n = 5$)

	Nominal concentration (ng/mL)	Measured concentration (mean ± S.D., ng/mL)	Precision R.S.D. (%)	Accuracy (%)
Intra-day	0.02	0.021 ± 0.001	6.46	106.73
	0.1	0.100 ± 0.005	5.45	99.53
	0.5	0.511 ± 0.024	4.74	102.16
	1.5	1.501 ± 0.078	5.20	100.08
Inter-day	0.02	0.020 ± 0.003	13.37	98.54
	0.1	0.100 ± 0.008	8.34	100.45
	0.5	0.491 ± 0.024	4.92	98.14
	1.5	1.492 ± 0.072	4.82	99.44

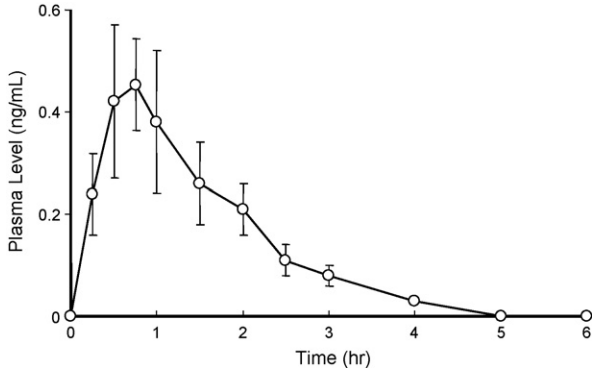

Fig. 4. Mean plasma concentration vs. time profiles of beraprost 12 male volunteers after the administration of 0.06 mg (0.02 mg × 3 tablets) dose of beraprost.

its terminal half-life ranging from 0.50 ± 0.21 to 0.91 ± 0.27 h independently of beraprost dose [3].

4. Conclusion

A rapid and sensitive method for determination of beraprost in human plasma by LC–MS/MS has been developed and validated and with a LLOQ of 0.02 ng/mL. The validation experiments have shown that the assay has good precision and accuracy over a concentration range (0.02–2 ng/mL), and no interferences caused by endogenous compounds were observed. This rapid, sensitive and robust assay enables a large number of samples processed for pharmacokinetic studies of beraprost in human plasma.

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