Journal of Chromatography B, 877 (2009) 527–532

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of eptifibatide concentration in human plasma utilizing the liquid chromatography–tandem mass spectrometry method

Jia Liu, Xiaotao Duan, Xiaoyan Chen, Dafang Zhong*

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 646 Songtao Road, Shanghai 201203, PR China

ARTICLE INFO

Article history:
Received 8 July 2008
Accepted 24 December 2008
Available online 6 January 2009

Keywords:
Eptifibatide
LC–MS/MS
Plasma

ABSTRACT

A sensitive and selective liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed to determine the concentration of eptifibatide in human plasma. Following protein precipitation, the analyte was separated on a reversed-phase C_{18} column. Acetonitrile:5 mM ammonium acetate:acetic acid (30:70:0.1, v/v/v) was used at a flow-rate of 0.5 mL/min with the isocratic mobile phase. An API 4000 tandem mass spectrometer equipped with a Turbo IonSpray ionization source was used as the detector and was operated in the positive ion mode. "Truncated" multiple reaction monitoring using the transition of m/z 832.6 → m/z 832.6 and m/z 931.3 → m/z 931.3 was performed to quantify eptifibatide and the internal standard (EPM-05), respectively. The method had a lower limit of quantification of 4.61 ng/mL for eptifibatide. The calibration curve was demonstrated to be linear over the concentration range of 4.61 – 2770 ng/mL. The intra- and inter-day precisions were less than 10.5% for each QC level, and the inter-day relative errors were 2.0%, 5.6%, and 2.8% for 9.22, 184, and 2490 ng/mL, respectively. The validated method was successfully applied to the quantification of eptifibatide concentration in human plasma after intravenous (i.v.) administration of a 270-μg/kg bolus of eptifibatide and i.v. administration of eptifibatide at a constant rate of infusion of 2 μg/(kg min) for 18 h in order to evaluate the pharmacokinetics.

1. Introduction

Eptifibatide (N^6-(aminoiminomethyl)-N^2-(3-mercapto-1-oxopropyl-L-lysylglycyl-L-α-aspartyl-L-tryptophyl-L-prolyl-L-cysteinamide, cyclic (1 → 6)-disulfide) (Fig. 1) is an antiplatelet drug that selectively blocks the platelet glycoprotein IIb/IIIa receptor and inhibits platelet aggregation. It is a cyclic heptapeptide derived from a protein found in the venom of the southeastern pygmy rattlesnake (*Sistrurus miliarus barbouri*). The drug is approved for use in patients presenting with non-ST-segment elevation acute coronary syndrome and those scheduled for percutaneous coronary intervention [1–3]. Eptifibatide exhibits a rapid on-rate to glycoprotein IIb–IIIa, and the off-rate is 10–15 s [4,5]. Due to its rapid binding and release, the plasma concentration of the agent is a critical determinant of receptor occupancy and inhibitory effects. In order to evaluate its pharmacokinetics, it is essential to establish an accurate, sensitive, and selective method for the quantification of eptifibatide concentration in plasma.

Liquid chromatography–mass spectrometry is recognized as a powerful analytical tool for the quantification of peptides in biological fluids. In the literature, several liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods using the selected reaction monitoring (SRM) process have been proposed for the measurement of cyclic peptide concentrations in plasma. Indeed, this method has been previously used to measure the concentration of cyclic peptides such as colistin (a cyclic heptapeptide and tripeptide side chain acylated at the N-terminus) [6] and melanotan-II (a cyclic heptapeptide, M_W: 1024 Da) [7]. Furthermore, in some cases, LC–MS methods using the SIM (selected ion monitoring) process have also been applied to measure the concentration of cyclic peptides such as cyclosporine A (a cyclic undecpeptide, M_W: 1202 Da) [8]. To date, however, there is no quantification method reported for eptifibatide.

Here we describe an LC–MS/MS method for the quantification of eptifibatide in human plasma that allows for a sensitive, rapid, and highly selective analysis.

2. Experimental

2.1. Chemicals and reagents

Eptifibatide and EPM-05 (internal standard, IS) were both kindly provided by Shenzhen Hanyu Pharmaceutical Factory Co. Ltd. (Guangdong, China). HPLC-grade acetonitrile and methanol

* Corresponding author. Tel.: +86 2150800738; fax: +86 2150800738.
E-mail address: zhongdf@china.com (D. Zhong).

1570-0232/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.12.060

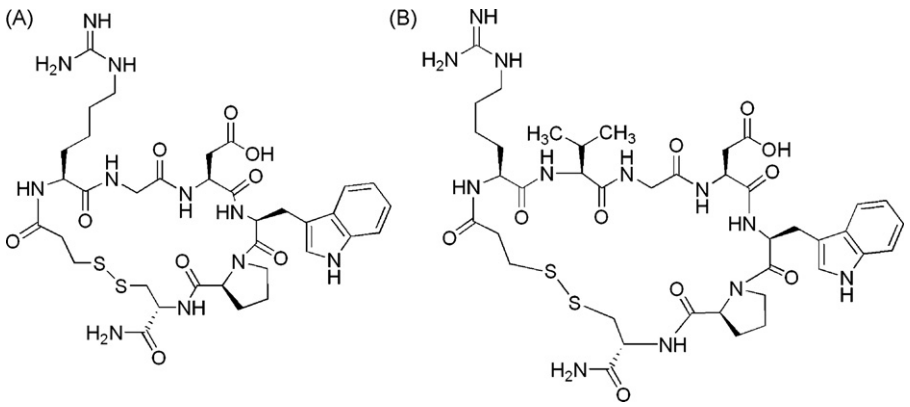

Fig. 1. Chemical structures of (A) eptifibatide and (B) EPM-05 (IS).

were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA), while HPLC-grade acetic acid and ammonium acetate were purchased from Tedia (Fairfield, OH, USA). Heparinized blank (drug-free) human plasma was obtained from the Shanghai Blood Donor Service (Shanghai, China). Ultrapure water (resistivity > 18.2 MΩ) was obtained by means of a MilliQ apparatus from Millipore (Millipore, Bedford, MA, USA).

2.2. Instrumentation and analytical conditions

A Shimadzu LC-20AD system with a SIL-HT autosampler (Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan) was used in the LC–MS/MS system. Separation of the analytes from the plasma was achieved on a Venusil ASB-C_{18} column (150 mm × 4.6 mm i.d., 5 μm; Angela Technologies Inc., Newark, DE, USA) with a Security-Guard C_{18} guard column (4 mm × 3.0 mm i.d.; Phenomenex, Torrance, CA, USA). A mobile phase of acetonitrile:5 mM ammonium acetate:acetic acid (30:70:0.1, v/v/v) at a flow-rate of 0.5 mL/min was employed under isocratic conditions.

An API 4000 triple-quadrupole mass spectrometer (Applied Biosystems, Concord, Ontario, Canada) was operated with a Turbo Ion Spray interface in positive ion mode. Analyst 1.4.1 software (Applied Biosystems) was used for the control of equipment, data acquisition, and analysis. For the optimization of MS/MS parameters, we made use of the software's auto-optimization feature and standard solutions of eptifibatide and the IS solution prepared in methanol were infused into the mobile phase (0.5 mL/min) at a flow-rate of 20 μL/min using a syringe pump (Harvard Apparatus, Holliston, MA, USA). Finally, the instrument was operated with the ion spray voltage set at +4.2 kV and the heater gas temperature at 400 °C. Additionally, we used a nebulizer gas (Gas 1) of 0.34 MPa, a heater gas (Gas 2) of 0.34 MPa, a curtain gas of 0.069 MPa, and a collision gas of 0.025 MPa. All gases used were nitrogen. The declustering potential (DP) was set at 100 V for both the analyte and the IS solution. Multiple reaction monitoring (MRM) was employed for data acquisition. The optimized "truncated" MRM fragmentation transitions were m/z 832.6 → m/z 832.6 with a collision energy (CE) of 20 eV for eptifibatide and m/z 931.3 → m/z 931.3 with a CE of 20 eV for the IS solution. The dwell time for each transition was 200 ms.

2.3. Preparation of standard and quality control (QC) samples

Stock solutions of eptifibatide at a concentration of 368.8 μg/mL were prepared by dissolving the accurately weighed reference substance in methanol. The stock solution was then serially diluted with methanol to give working solutions at concentrations of 0.023, 0.046, 0.138, 0.369, 0.922, 1.84, 4.61, and 13.8 μg/mL for eptifibatide. The other stock solution was independently diluted in a similar way to achieve quality control (QC) solutions at concentrations of 0.046, 0.922, and 12.4 μg/mL. IS solution (100 ng/mL) was prepared by diluting 50.77 μg/mL of EPM-05 with methanol. All the solutions were kept at 4 °C and were brought to room temperature before use.

Both the calibration standard samples and the quality control samples, which were used in the pre-study validation and during the pharmacokinetic study, were prepared by spiking 200 μL of blank plasma with 40 μL of the working solution.

2.4. Sample preparation

A 40-μL aliquot of the IS solution (EPM-05, 100 ng/mL) and 240 μL of methanol were added to 200 μL of plasma samples. The new sample was vortex-mixed for 1 min, followed by centrifugation at 11,374 × g for 5 min. The upper phase was transferred to a clean glass tube and evaporated to dryness at 40 °C under a stream of nitrogen in the TurboVap evaporator (Zymark, Hopkinton, MA, USA). The residue was reconstituted in 150 μL of the mobile phase and vortex-mixed for 1 min. A 20-μL aliquot of the reconstituted extract was injected for LC–MS/MS analysis.

2.5. Method validation

The validation experiments were designed according to "Guidance for Industry-Bioanalytical Method Validation," recommended by the US Food and Drug Administration (FDA) [9], with consideration of the intended application of the assay for sample analysis.

Specificity of the method was evaluated by assaying human blank plasma samples from six different donors and the lower limit of quantification (LLOQ) samples, respectively. LLOQ was defined as the lowest concentration of analytes determined with acceptable precision and accuracy (six replicates with relative standard deviation (R.S.D.) below 20% and a relative error (RE) within ±20%). Moreover, the analytes' responses at this concentration level should be >5 times the baseline noise.

Linearity was assessed by plotting calibration curves in human plasma in duplicate in three separate runs. The curves were fitted by a linear weighted ($1/x^2$) least squares regression method through measurement of the peak-area ratio of the analytes to the IS.

To evaluate the precision and accuracy of the method, QC samples at three concentration levels (9.22, 184, and 2490 ng/mL for eptifibatide) were analyzed in six replicates on three validation days. The assay precision was calculated by using the R.S.D. and a one-way analysis of variance (ANOVA).

R.S.D. separates out the sources of variance due to within- and between-run factors. The assay accuracy was expressed as the RE, or (observed concentration − nominal concentration)/(nominal con-

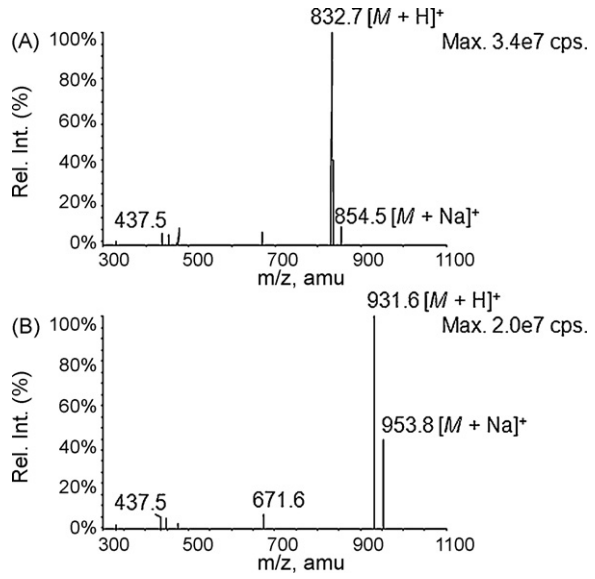

Fig. 2. Full scan mass spectra of (A) eptifibatide and (B) EPM-05 (IS).

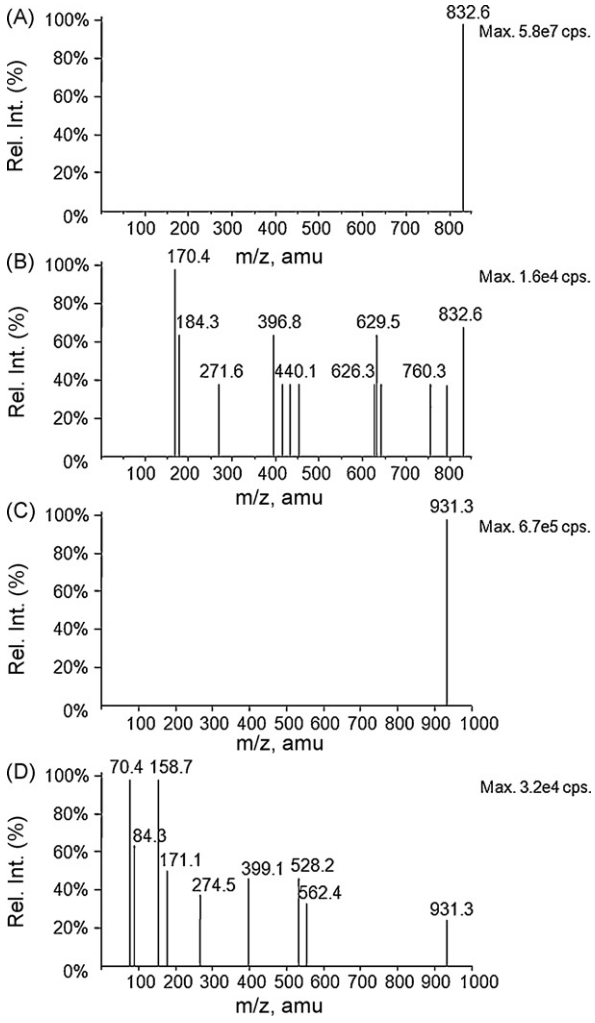

Fig. 3. MS^2 mass spectra of (A) eptifibatide with a collision energy (CE) of 20 eV; (B) eptifibatide with a collision energy (CE) of 70 eV; (C) EPM-05 (IS) with a collision energy (CE) of 20 eV; and (D) EPM-05 (IS) with a collision energy of 70 eV.

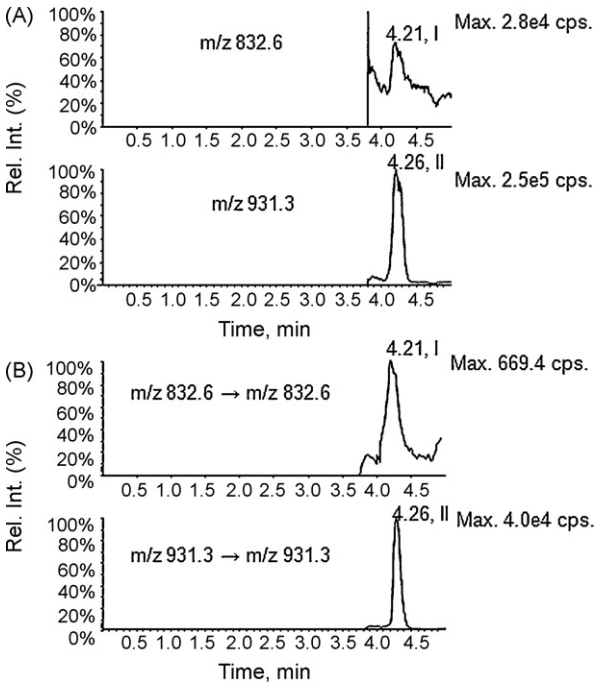

Fig. 4. Representative chromatograms for eptifibatide (I) and IS (EPM-05, II) in human plasma samples: (A) the SIM chromatograms of blank plasma spiked with eptifibatide (9.22 ng/mL) and EPM-05 (IS, 20.0 ng/mL); (B) the truncated MRM chromatograms of blank plasma spiked with eptifibatide (4.61 ng/mL) and EPM-05 (IS, 20.0 ng/mL).

centration) × 100%. The accuracy was required to be within ±15%, and the intra- and inter-day precisions were not to exceed 15%.

To evaluate the precision and accuracy of the method in the sample dilution process, QC samples at two concentration levels (9220 and 92,200 ng/mL for eptifibatide) were respectively, diluted 10-fold and 100-fold before being analyzed in six replicates on the first day of pharmacokinetic study.

The recoveries of eptifibatide at three QC levels ($n = 6$) were determined by comparing peak-area ratios of the analytes to the IS in samples that were spiked with the analytes prior to protein precipitation and samples to which the analytes were added post-protein precipitation. The IS solution was added to both sets of samples post-protein precipitation. The recovery of the IS was determined in a similar way, using the QC samples at medium concentration as a reference.

According to the method described by Matuszewski et al. [10], we assessed the matrix effects (MEs), or whether the potential ion suppression or enhancement owing to the co-eluting matrix components existed in the present experiment. The corresponding peak areas of the analyte from the spike-after-protein precipitation samples at low and high concentration levels were then compared to those of the standard solution at the same concentration in the mobile phase.

The stabilities of the eptifibatide working solutions were determined after exposure at 4 °C for 3 months. These results were compared with the working solutions newly obtained. The analytes were considered stable in the methanol when the intensities ranged between 85% and 115% of the initial solutions. The stability of eptifibatide in human plasma was evaluated by analyzing replicates ($n = 3$) of plasma samples that were exposed to different conditions (time and temperature) at concentrations of 9.22 and 2490 ng/mL. These results were compared with those obtained for freshly prepared plasma samples. The analytes were considered stable in the biological matrix when 85–115% of the initial concentrations were found. The short-term stability was determined after exposure of

the spiked samples at 22 °C for 2 h and placement of the ready-to-inject samples (after extraction) on the autosampler rack (22 °C) for 24 h. The freeze/thaw stability was evaluated after three complete freeze/thaw cycles (−20 to 22 °C) on consecutive days.

2.6. Application to a clinical pharmacokinetic study

The validated method was used to determine the plasma concentrations of eptifibatide in a pharmacokinetic study of the drug. Nine Chinese healthy male volunteers received intravenous (i.v.) administration of a 270-μg/kg bolus dose of eptifibatide. The blood samples were drawn at baseline (30 min before the study drug bolus) and at 0.083, 0.17, 0.25, 0.50, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, and 12 h after drug administration. Another nine Chinese healthy male volunteers received i.v. administration of eptifibatide at a constant rate of infusion of 2 μg/(kg min) for 18 h. The blood sampling was carried out during the drug infusion at 2, 6, and 12 h. Blood samples were also taken at 0.0, 0.083, 0.17, 0.25, 0.50, 0.45, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, and 12 h after drug infusion. The study protocol was approved by the Human Investigation Ethical Committee and blood sampling was carried out at the First Affiliated Hospital of Third Military Medical University. Plasma samples were obtained by centrifugation at 2000 × *g* for 10 min and frozen at −80 °C until analysis.

3. Results and discussion

3.1. LC–MS/MS optimization

Under our experiment conditions, the soft ionization process in the ESI source produced the protonated molecule $[M+H]^+$ of eptifibatide at *m*/*z* 832.6 as the base peak, while the peak at *m*/*z* 854.6 corresponded to $[M+Na]^+$. Due to only one basic arginine moiety present in the structure of eptifibatide, only a single-charged ion was observed in the acidic mobile phase.

When quantifying peptides or proteins, SRM [6,7] or SIM [8] has often been employed with satisfactory sensitivity. However, in some cases, due to the structures or physicochemical properties of the analytes, common SRM and SIM procedures are not enough to meet the requirements. In a recent study related to quantifying human insulin-like growth factor-1, a modified MRM process was used. This mode selects several precursor ions and characteristic product ions generated by collision-induced dissociation (CID) without including dissociation, thereby improving the sensitivity [11]. In our work, owing to the cyclic structure of eptifibatide, the protonated molecule was stable with a CE of 20 eV and no noticeable product ion. However, upon increasing the CE to 70 eV, too many fragments were obtained. Furthermore, no appropriate product ion could be selected. It should also be noted that traditional SIM was not suitable to quantify the analyte. Under SIM the signal-to-noise ratio of etifibatide at 9.22 ng/mL was only 4.9 and an interfering peak could easily be seen. Finally, the CE was set at 20 eV and SRM at *m*/*z* 832.6 → *m*/*z* 832.6 was used for quantification. Under these conditions, although the relative intensity was reduced, the sensitivity was improved; the LLOQ decreased from 9.22 to 4.61 ng/mL, possibly due to the dissociation of interfering compounds.

In our case, EPM-05 (a structure analog of eptifibatide) was used as the internal standard because of the similar behavior of LC and MS to that of eptifibatide. The base peak $[M+H]^+$ ion at *m*/*z* 931.3 in the Q1 full scan mode was selected as the precursor ion. Similar to the process employed to quantify eptifibatide, multiple reaction monitoring at *m*/*z* 931.3 → *m*/*z* 931.3 with a CE of 20 eV was used for quantification.

The full scan mass spectra of eptifibatide and the IS are shown in Fig. 2. The MS^2 mass spectra of eptifibatide and the IS at different collision energies are shown in Fig. 3. The SIM chromatograms of blank plasma spiked with eptifibatide (9.22 ng/mL) and the IS (20.0 ng/mL) are shown in Fig. 4(A). The truncated MRM chromatograms of blank plasma spiked with eptifibatide (4.61 ng/mL) and the IS (20.0 ng/mL) are shown in Fig. 4(B).

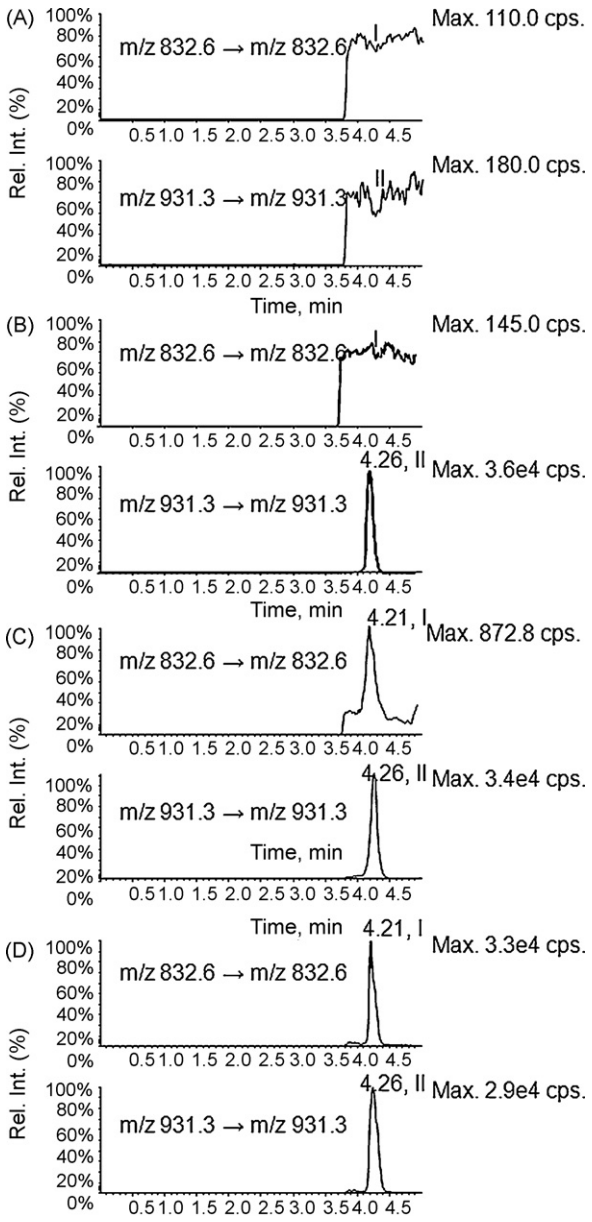

Fig. 5. Representative chromatograms for eptifibatide (I) and IS (EPM-05, II) in human plasma samples: (A) the truncated MRM chromatograms of the blank plasma sample; (B) the truncated MRM chromatograms of blank plasma spiked with EPM-05 (IS, 20.0 ng/mL); (C) the truncated MRM chromatograms of blank plasma spiked with eptifibatide (9.22 ng/mL) and EPM-05 (IS, 20.0 ng/mL); and (D) the truncated MRM chromatograms of a plasma sample taken from a volunteer 6 h into i.v. administration of eptifibatide at a constant rate of infusion of 2 μg/(kg min) (899 ng/mL).

In our chromatographic condition, the analyte and the IS had suitable retention times. The retention times for eptifibatide and the IS were approximately 4.21 and 4.26 min, respectively.

In the experiment, no significant carry-over was observed by injecting an extract of blank matrix immediately following the highest calibration standard (upper limit of quantitation). As shown in Fig. 5(B), the IS did not influence the quantification of eptifibatide.

Table 1
Accuracy and precision for the analysis of eptifibatide in human plasma (in pre-study validation).

Nominal conc. (ng/mL)	Intraday measured conc. (ng/mL)			Interday validation summary			
	Day 1	Day 2	Day 3	Mean (ng/mL)	S.D. (ng/mL)	R.S.D. (%)	Relative error (%)
9.22	8.97	7.84	8.41	9.40	0.898	10.5	2.0
	9.84	8.69	10.1				
	8.69	9.97	8.80				
	10.0	8.78	9.86				
	11.4	8.74	8.65				
	10.1	10.2	10.2				
Mean (ng/mL)	9.83	9.04	9.34				
S.D. (ng/mL)	0.960	0.887	0.803				
Relative error (%)	6.7	−2.0	1.3				
R.S.D. (%)	9.8	9.8	8.6				
184	204	201	196	194	10.8	4.9	5.6
	209	198	183				
	207	203	190				
	185	199	197				
	191	179	196				
	195	165	200				
Mean (ng/mL)	199	191	194				
S.D. (ng/mL)	9.63	15.3	6.15				
Relative error (%)	7.9	3.7	5.3				
R.S.D. (%)	4.9	8.1	3.2				
2490	2670	2550	2520	2559	121	8.3	2.8
	2360	2480	2590				
	2510	2570	2540				
	2420	2560	2630				
	2480	2550	2770				
	2370	2710	2790				
Mean (ng/mL)	2468	2570	2640				
S.D. (ng/mL)	115	75.6	115				
Relative error (%)	−0.9	3.2	6.0				
R.S.D. (%)	4.7	2.9	4.4				

3.2. Method validation

3.2.1. Linearity of calibration standards

The plotted calibration curves and correlation coefficients >0.99 confirmed that the calibration curves were linear over the concentration range of 4.61–2770 ng/mL for eptifibatide. A typical standard curve for eptifibatide was as follows: $y = 1.02 \times 10^{-3}, x + 5.59 \times 10^{-3}$, $r = 0.9987$, where y represents the ratio of the analyte peak area to that of the IS and x represents the plasma concentration of the analyte.

3.2.2. Assay specificity and LLOQ

Three typical truncated MRM chromatograms from the quantification of eptifibatide in human plasma are shown in Fig. 5. No interfering peak was observed in the blank plasma (Fig. 5(A)). The truncated MRM chromatograms of blank plasma spiked with eptifibatide (9.22 ng/mL) and the IS (20.0 ng/mL) are shown in Fig. 5(C). A sample from a volunteer taken 6 h into an infusion of 2.0 μg/(kg min) of eptifibatide is shown in Fig. 5(D). The chromatograms for the drugs and the IS were free from endogenous matrix interference at their respective retention times.

For eptifibatide, the present LC–MS/MS method offered an LLOQ of 4.61 ng/mL with an accuracy of 2.4% in terms of RE and a precision of 10.0% in terms of R.S.D. ($n = 6$).

3.2.3. Precision and accuracy

The intra- and inter-day precision and accuracy were calculated by an analysis of variances based on replicate analyses (3 days, three concentrations, each $n = 6$) of QC samples. The results are summarized in Table 1. In this study, the intra- and inter-day precisions were less than 10.5% for each QC level. The inter-day relative errors were 2.0%, 5.6%, and 2.8% for the three concentrations, respectively. These data indicated reproducible LC–MS/MS results, and that the assay was accurate and reliable. The accuracy and precision data are shown in Table 1.

3.2.4. Recovery and stability

The recovery results of eptifibatide, determined at three concentrations (9.22, 184, and 2490 ng/mL), were 101 ± 5.0%, 95.5 ± 2.8%, and 103 ± 4.5% ($n = 6$), respectively. The recovery of the IS was shown to be 93.9 ± 1.2% ($n = 6$). The recovery data are shown in Table 2.

The results of stability experiments showed that eptifibatide was stable for 3 months in methanol, for 12 h after preparation at 22 °C, for 2 h at 22 °C, and after three freeze/thaw cycles (−20 to 22 °C) on consecutive days, as the RE values were within ±15% for both the low and high concentrations.

Table 2
Recovery of eptifibatide and EPM-05 in human plasma (two concentrations, $n = 6$).

Recovery (%)	Conc. of eptifibatide (ng/ml)			Conc. of EPM-05 (ng/ml)
	9.22	184	2490	20.0
	95.1	96.7	98.5	92.1
	106	90.4	101	95.6
	97.6	94.1	99.2	93.8
	104	96.8	103	94.1
	96.7	96.4	108	94.7
	106	98.4	109	93.3
n	6	6	6	6
Mean (%)	101	95.5	103	93.9
S.D. (%)	5.0	2.8	4.5	1.2

Table 3
Matrix effect of eptifibatide and EPM-05 in human plasma (three concentrations, $n = 5$).

Matrix factor* (%)	Conc. of eptifibatide (ng/ml)		Conc. of EPM-05 (ng/ml)
	9.22	2490	20.0
	123	92.0	105
	114	95.0	102
	100	102	106
	120	92.1	110
	107	92.2	110
n	5	5	5
Mean (%)	113	94.7	107
S.D. (%)	9.5	4.3	3.4

* Matrix factor = (peak area of the analyte from the spike-after-protein precipitation sample)/(peak area of the standard solution) × 100%.

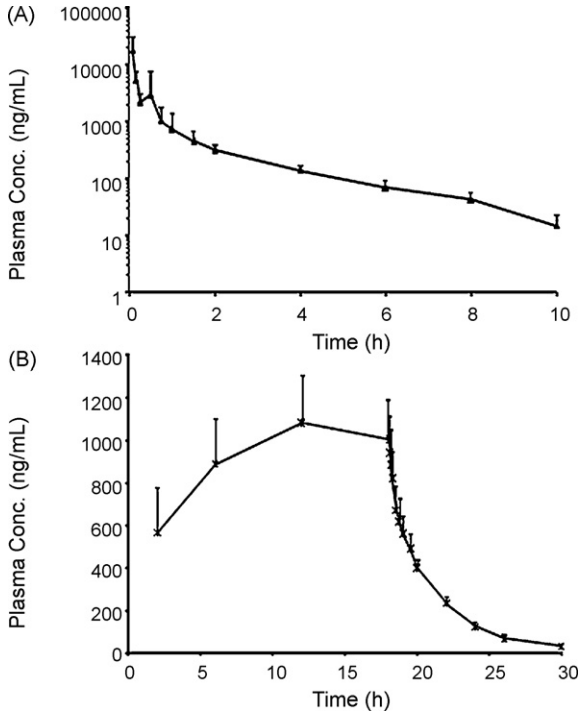

Fig. 6. Mean plasma concentration–time curve of eptifibatide in healthy subjects: (A) i.v. administration of a 270-μg/kg bolus dose of eptifibatide ($n = 9$, mean ± S.D.) and (B) i.v. administration of eptifibatide at a constant rate of infusion of 2 μg/(kg min) ($n = 9$, mean ± S.D.).

When the results are taken into consideration, it can be said that eptifibatide samples can be stored and prepared under routine laboratory conditions without special attention.

3.2.5. Matrix effect

The matrix factors of eptifibatide, determined at two concentrations (9.22 and 2490 ng/mL), were 113 ± 9.5% and 94.7 ± 4.3% ($n = 5$), respectively. The matrix factor of EPM-05 was 107 ± 3.4%. The results showed that using the present LC–MS/MS method, the MEs of both eptifibatide and EPM-05 could be ignored. The recovery data are shown in Table 3.

3.3. Application of the method to a pharmacokinetic study in healthy subjects

The validated analytical method was applied to the assay of eptifibatide in human plasma in a pharmacokinetic study of the drug. The plasma samples were processed based on the proposed extraction protocol for the quantification of eptifibatide. Mean plasma concentration versus time profile is presented in Fig. 6. This analytical method was sensitive enough to monitor the concentration of eptifibatide for up to 8 h in all subjects and up to 10 h in 88.9% of the subjects who received i.v. administration of a bolus dose of 270 μg/kg of eptifibatide. Furthermore, this method was sensitive enough to monitor the concentration of eptifibatide for up to 30 h in all subjects who received i.v. administration of eptifibatide at a constant rate of infusion of 2.0 μg/(kg min) for 18 h.

4. Conclusion

A rapid and sensitive LC–MS/MS method was developed and validated for the quantification of eptifibatide in human plasma. The method showed a high recovery and a negligible ME. The LLOQ was 4.61 ng/mL for eptifibatide using 200 μL of human plasma. In summation, it can be said that this method was simple and robust, and was utilized to support clinical pharmacokinetic studies of eptifibatide.

References

[1] D.J. Schneider, A. Aggarwal, Expert Rev. Cardiovasc. Ther. 2 (2004) 903.
[2] D.D. Gretler, Clin. Ther. 25 (2003) 2564.
[3] Millennium Pharmaceuticals Inc., INTEGRILIN® (eptifibatide) injection product information, Rev. 3/07.
[4] D. Phillips, R. Scarborough, Am. J. Cardiol. 80 (1997) B11.
[5] S. Moussa, J. Bennet, Drugs Future 21 (1996) 1141.
[6] Z. Ma, J. Wang, J.P. Gerber, R.W. Milne, J. Chromatogr. B 862 (2008) 205.
[7] S. Hatziieremia1, N. Kostomitsopoulos, V. Balafas, C. Tamvakopoulos, Rapid Commun. Mass Spectrom. 21 (2007) 2431.
[8] N. Ansermot, M. Fathi, J.L. Veuthey, J. Desmeules, D. Hochstrasser, R. Serge, J. Chromatogr. B 857 (2007) 92.
[9] US Food and Drug Administration, Guidance for Industry: Bioanalytical Method Validation, 2001.
[10] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[11] M. Bredehöft, W. Schänzer, M. Thevis, Rapid Commun. Mass Spectrom. 22 (2008) 477.