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Simultaneous determination of fixed dose combination of nebivolol and valsartan in human plasma by liquid chromatographic-tandem mass spectrometry and its application to pharmacokinetic study

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Abstract

A rapid, sensitive and accurate liquid chromatographic-tandem mass spectrometry method is described for the simultaneous determination of nebivolol and valsartan in human plasma. Nebivolol and valsartan were extracted from plasma using acetonitrile and separated on a C18 column. The mobile phase consisting of a mixture of acetonitrile and 0.05 mM formic acid (50:50 v/v, pH 3.5) was delivered at a flow rate of 0.25 ml/min. Atmospheric pressure ionization (API) source was operated in both positive and negative ion mode for nebivolol and valsartan, respectively. Selected reaction monitoring mode (SRM) using the transitions of m/z 406.1 → m/z 150.9; m/z 434.2 → m/z 179.0 and m/z 409.4 → m/z 228.1 were used to quantify nebivolol, valsartan and internal standard (IS), respectively. The linearity was obtained over the concentration range of 0.01–50.0 ng/ml and 1.0–2000.0 ng/ml and the lower limits of quantitation were 0.01 ng/ml and 1.0 ng/ml for nebivolol and valsartan, respectively. This method was successfully applied to the pharmacokinetic study of fixed dose combination (FDC) of nebivolol and valsartan formulation product after an oral administration to healthy human subjects.
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Keywords: Nebivolol; Valsartan; LC–API-MS–MS

1. Introduction

Nebivolol hydrochloride is chemically known as α,α′-[iminobis(methylene)]bis[6-flouro-3,4-dihydro-2H-1-benzopyran-2-methanol]hydrochloride [1], it is a highly selective β_1-blocker with nitric oxide-mediated vasodilatory actions and beneficial effects on vascular endothelial function. It has been clinically used for the treatment of hypertension and chronic heart failure [2]. And valsartan is chemically described as *N*-(1-oxopentyl)-*N*-[[2′-(1H-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-L-valine [3], it is a nonpeptide angiotensin II AT_1-receptor antagonist and very well-tolerating hypertension by specifically blocking the action of angiotensin-II on the angiotensin Type-1 receptor. It has been clinically used for the treatment of hypertension and heart failure [4]. Structures of the both analytes and IS are shown in Fig. 1.

For the pharmacokinetic study on FDC of nebivolol and valsartan formulation product in human subjects, an analytical method with simplicity and high sensitivity was required in our laboratory. A recent survey revealed that few individual methods were available for the determination of nebivolol and valsartan in biological samples. For nebivolol, this involved HPLC, radioimmunoassay (RIA) method [5] and liquid chromatographic-tandem mass spectrometry (LC–MS–MS) with electrospray ionization (ESI) and solid phase extraction (SPE) [6]. Valsartan was also analyzed by HPLC with fluorimetric detection [7–9] and HPLC with ultraviolet (UV) detection and SPE [10]. Although various analytical methods have been developed for the quantitation of nebivolol and valsartan individually from human plasma. There has been no report in the literature on the simultaneous determination of nebivolol and valsartan in human plasma which is applicable to pharmacokinetic studies on FDC. To our knowledge the proposed method is the only method for the simultaneous determination of nebivolol and valsartan in human plasma by using LC–MS–MS with API source and protein precipitation extraction method.

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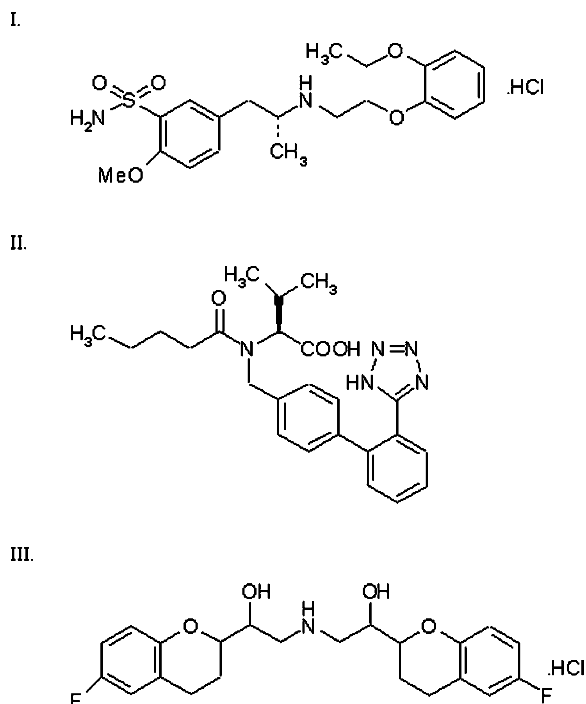

Fig. 1. Chemical structures of (I) nebivolol hydrochloride; (II) valsartan and (III) tamsulosin hydrochloride (internal standard).

In API source, the turbo electrospray ionization (ESI) offers some advantages over APCI source in terms of less background noises and suitability for small molecular compounds. Taking into consideration the low levels of nebivolol and valsartan in plasma, LC–MS–MS method with simple and economic sample preparation method is the first choice for our purpose. The extraction procedure described by Hoja et al. is cumbersome and involves multi step extraction; basic extraction, acid back-extraction, acid wash and basic re-extraction [11], the extraction procedure described by Ramakrishna et al. for nebivolol is a liquid–liquid extraction method with two different organic solvents [6] and the extraction procedure described by Perez et al. for valsartan is a SPE method by using C8 cartridge [10]. On the contrary, a simple and single step extraction of nebivolol and valsartan with acetonitrile has been reported in the present method. Tamsulosin hydrochloride was used as IS to compensate the changes in sample concentration during sample preparation [12]. Besides, a less sample mass onto the column is often preferred in ordinary laboratories for the benefits of both system maintenance and re-injections of the extracted sample when only a limited sample is available.

This paper describes a simple, specific and highly sensitive LC–MS–MS method with API source in SRM mode for the determination of nebivolol and valsartan in human plasma. The proposed method was validated in terms of matrix effect, selectivity, sensitivity, linearity, accuracy, precision and stability of analyte in plasma and mobile phase, and successfully applied to the pharmacokinetic study of FDC of nebivolol and valsartan formulation product after an oral administration to healthy human subjects. In addition, to our knowledge, this is the first, simple and highly validated LC–MS–MS the state-of-the-art technique for the simultaneous determination of nebivolol and valsartan in human plasma.

2. Experimental

2.1. Chemicals and reagents

The nebivolol hydrochloride standard and nebivolol 5 mg (Nebicard-5) tablet were purchased from Torrent Laboratories Ltd. (Ahmedabad, India). The valsartan standard and valsartan 80 mg (Valent-80) capsule were purchased from Lupin Laboratories Ltd. (Mumbai, India). The IS was procured from CIPLA Ltd. (Mumbai, India). Acetonitrile (LiChrosolv®) was purchased from Merck (Mumbai, India). Formic acid and ammonium acetate were procured from Sigma–Aldrich (Bangalore, India). HPLC grade water was acquired from an in-house water purification system (Milli-Q, Molsheim, France) and used throughout this study. The HPLC mobile phase and sample aliquots were filtered through a 0.45 μm and 0.2 μm Nylon-66 filters (Aligent Technologies, CA, USA), respectively before use.

2.2. Instrumentation

A liquid chromatographic system consisting of LC-20AD pump; SIL-20AC auto-sampler and CTO-10ASvp column oven (Shimadzu, Kyoto, Japan) were used for the separation. The LC–MS–MS system consisting of atmospheric pressure ionization source (API-2000) with triple quadrupole tandem mass spectrometer (AB Sciex Instruments, Foster, CA) was used for quantitative determination of nebivolol and valsartan in human plasma. Data integration was performed with Analyst 1.4.1 software version (AB Sciex Instruments, Foster, CA).

2.3. Chromatographic conditions

LC separation was performed on a Gemini-C18 column (50 mm × 2.0 mm i.d., 3 μm, Phenomenex, Torrance, CA, USA) with a Security Guard C18 guard column (4 mm × 3.0 mm i.d., Phenomenex, Torrance, CA, USA). The mobile phase consisting of a mixture of acetonitrile and 0.05 mM formic acid buffer (50:50 v/v, pH 3.5) was delivered at a flow rate of 0.25 ml/min. The column temperature was maintained at 20 °C. The injection volume was 10.0 μl.

2.4. Mass spectrometric conditions

A triple quadrupole mass spectrometer (MS–MS) was used with API source and channel electron multiplier (CEM) detector. In the API source, the turbo electrospray ionization (ESI) interface was operated on both the positive and negative modes alternatively for the better ionization of nebivolol, IS and valsartan, respectively. Zero air was used as an ion source gas and heater gas. The ultra high purity nitrogen was served as curtain gas for the effect of collisional induced disassociation (CID) in the curtain plate region and the collisional gas for the effect of collisional activated disassociation (CAD) in the

Table 1
Tandem mass spectrometer main working parameters

Parameters	Values for nebivolol/tamsulosin	Values for valsartan
Source temperature (TEM) (°C)	450.0	450.0
Ion source gas (gas 1) (psi)	35.0	35.0
Heater gas (gas 2) (psi)	55.0	55.0
Curtain gas (CUR) (psi)	28.0	28.0
Collision gas (CAD) (psi)	6.0	6.0
Ion spray voltage (IS) (V)	5500.0	−4500.0
Collision energy (CE) (V)	42.5	−32.0
Declustering potential (DP) (V)	50.0	−25.0
Focusing potential (FP) (V)	400.0	−400.0
Entrance potential (EP) (V)	10.0	−10.0
Collision cell entrance potential (CEP) (V)	31.0	−28.4
Collision cell exit potential (CXP) (V)	2.1	−6.5
Dwell time per transition (ms)	100.0	100.0
Mode of analysis	(+) ve	(−) ve
Ion transition (*m/z*)	406.1 → 150.9/409.4 → 228.1	434.2/179.0

collision cell. For the simultaneous determination of precursor ions and fragment ions of the analytes and IS were done using selected reaction monitoring (SRM) mode. The transitions selected were *m/z* 406.1 → *m/z* 150.9; *m/z* 434.2 → *m/z* 179.0 and *m/z* 409.4 → *m/z* 228.1 for nebivolol, valsartan and IS, respectively. The main working parameters of the mass spectrometer are summarized in Table 1.

2.5. Preparation of calibration standards and quality control samples

Stock solutions of nebivolol, valsartan and IS were individually prepared at 100.0 μg/ml in acetonitrile. The stock solution of nebivolol and valsartan were further diluted with water to give a series of standard solutions with concentration of 0.1, 0.25, 0.5, 0.75, 1.0, 5.0, 10.0, 50.0, 100.0, 200.0, 400.0, 500.0 ng/ml and 10.0, 25.0, 50.0, 100.0, 250.0, 500.0, 1000.0, 2500.0, 5000.0, 10,000.0, 15,000.0, 20,000.0 ng/ml, respectively. A solution containing 600.0 ng/ml of IS was prepared with acetonitrile. Calibration standards of nebivolol and valsartan (0.01, 0.025, 0.05, 0.075, 0.1, 0.5, 1.0, 5.0, 10.0, 20.0, 40.0, 50.0 ng/ml and 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0, 1000.0, 1500.0, 2000.0 ng/ml, respectively) were prepared by spiking appropriate amount of the standard solutions in blank plasma obtained from healthy human volunteers. The quality control (QC) samples were prepared using the pooled plasma at concentrations of 0.025, 5.0, 40.0 ng/ml for nebivolol and 2.5, 250.0, 1500.0 ng/ml for valsartan. The spiked samples were then treated following the sample preparation procedure as indicated in Section 2.6.

2.6. Sample preparation

In 2.0 ml polyethylene centrifuge tube, 200.0 μl of each plasma sample was transferred and 400.0 μl of HPLC grade acetonitrile which contained 240.0 ng/ml of IS was added. The contents of the centrifuge tubes were briefly mixed by vortexing for 1 min and centrifuged at 14,000 rpm for 10 min to separate the supernatant liquid. The aliquots were filtered through 0.2 μm membrane and injected onto the LC–MS–MS system.

2.7. Method validation

Validation runs were conducted on three separate days. Each validation run consisted of a set of spiked standard samples at 12 concentrations over the concentration range (each in triplicate), QC samples at three concentrations ($n = 6$, at each concentration), blank, stability and freeze-thaw samples. Standard samples were analyzed at the beginning of each validation run and other samples were distributed randomly throughout the run. The results from QC samples in three runs were used to evaluate the accuracy and precision of the method developed. Concentrations of the analyte in plasma samples were determined by back-calculation of the observed peak area ratios of the analyte and IS from the best-fit calibration curve using a weighted ($1/x$) linear regression. During routine analysis, each analytical run included a set of standard samples, a set of QC samples in duplicate and plasma samples to be determined.

The matrix effect was investigated by collecting blank plasma from 12 different sources and spiked with low and high QC concentration of nebivolol, valsartan and IS. After the extraction, the sample aliquots were analyzed and the peak areas of the spiked QC concentrations of 12 different sources were compared.

The extraction recovery of nebivolol, valsartan and IS were determined at low, medium and high QC concentrations by comparing the responses from plasma samples spiked before extraction with those from plasma samples extracted and spiked after extraction.

Sample stability in terms of freeze-thaw stability, short-term room temperature and long-term stability were tested by analyzing QC samples at concentrations of 0.025, 5.0, 40.0 ng/ml for nebivolol and 2.5, 250.0, 1500.0 ng/ml for valsartan. The freeze-thaw stability (24 h at −20 °C for four cycles), short-term stability (24 h, room temperature) and long-term stability (45 days at −20 °C) were calculated. The stock solution standards of nebivolol, valsartan and IS were prepared in acetonitrile, stored at −20 °C for 30 days and the stability was evaluated. The post-preparative stability was tested by comparing after-day analysis with the first-day analysis.

2.8. Application of the LC–MS–MS method

The LC–MS–MS method developed was successfully applied to the pharmacokinetic study of FDC of nebivolol and valsartan in healthy human subjects. Twelve healthy human volunteers aged 21–27 years were admitted in Bioequivalence Study Center (Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India). After an overnight fast (12 h), each volunteer was given either single dose of test preparation (FDC capsule containing 5 mg of nebivolol as a film coated tablet 80 mg of valsartan as granules) or reference preparation nebivolol 5 mg (Nebicard-5) tablet and valsartan 80 mg (Valent-80) capsule with 240.0 ml of water. No food was allowed until 3 h after oral administration of the doses. About 5.0 ml of blood samples were collected from the forehand vein into heparinized tubes before (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, 24, 36 and 48 h after dosing. Plasma was separated by centrifugation at 3000 × *g* for 10 min and kept frozen at −20 °C until analysis.

3. Results and discussion

3.1. Method development

Protein precipitation technique was used as an extraction method for sample preparation in this work. Protein precipitation technique can be helpful in producing a spectroscopically clean sample and avoiding endogenous substances in plasma with the analytes and IS onto the column and MS system. Clean samples are essential for minimizing ion suppression and matrix effect in LC–MS–MS analyses. Two organic solvents, methanol, acetonitrile and their mixtures in different ratios were evaluated. Finally acetonitrile was found to be optimal, which can produce a clean chromatogram for a blank plasma sample and yield the highest recovery for the analytes from the human plasma.

The chromatographic conditions, especially the composition of mobile phase, were optimized through several trials to achieve good resolution and symmetric peak shapes of analytes as well as short run time. It was found that a mixture of acetonitrile and 0.05 mM formic acid (50:50 v/v, pH 3.5) could achieve our purpose and was finally adopted as the mobile phase for the chromatographic separation.

An IS is necessary for determination of analytes in biological samples. For an LC–MS–MS analysis, utilization of stable isotope-labeled drugs as IS proves to be helpful when significant matrix effect occurs. However, there are also many problems with the use of stable isotope-labeled IS. The major problems involve inadequate isotopic purity and stability, which often impose unfavorable impact on highly sensitive quantitative analyses. In initial stage of our work, several compounds were tried to find a suitable IS and finally tamsulosin was found to be optimal for our work. Clean chromatograms were obtained and no significant matrix effect was found in the recovery of nebivolol and valsartan.

For the quantitation of nebivolol and valsartan in human plasma, some parameters related with tandem mass spectrometry were investigated. Based on our experiences, API was

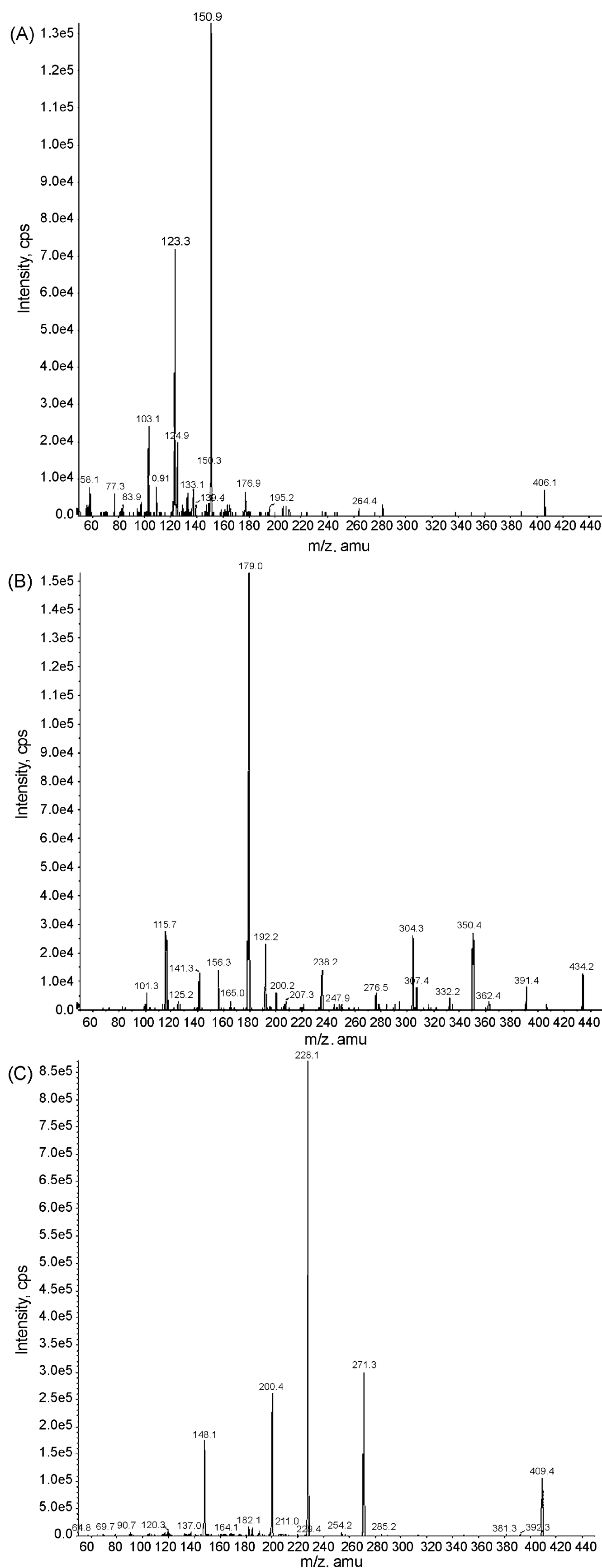

Fig. 2. Profile mass spectral data of (A) nebivolol $[M+H]^+$; (B) valsartan $[M-H]^-$ and (C) tamsulosin $[M+H]^+$ (internal standard).

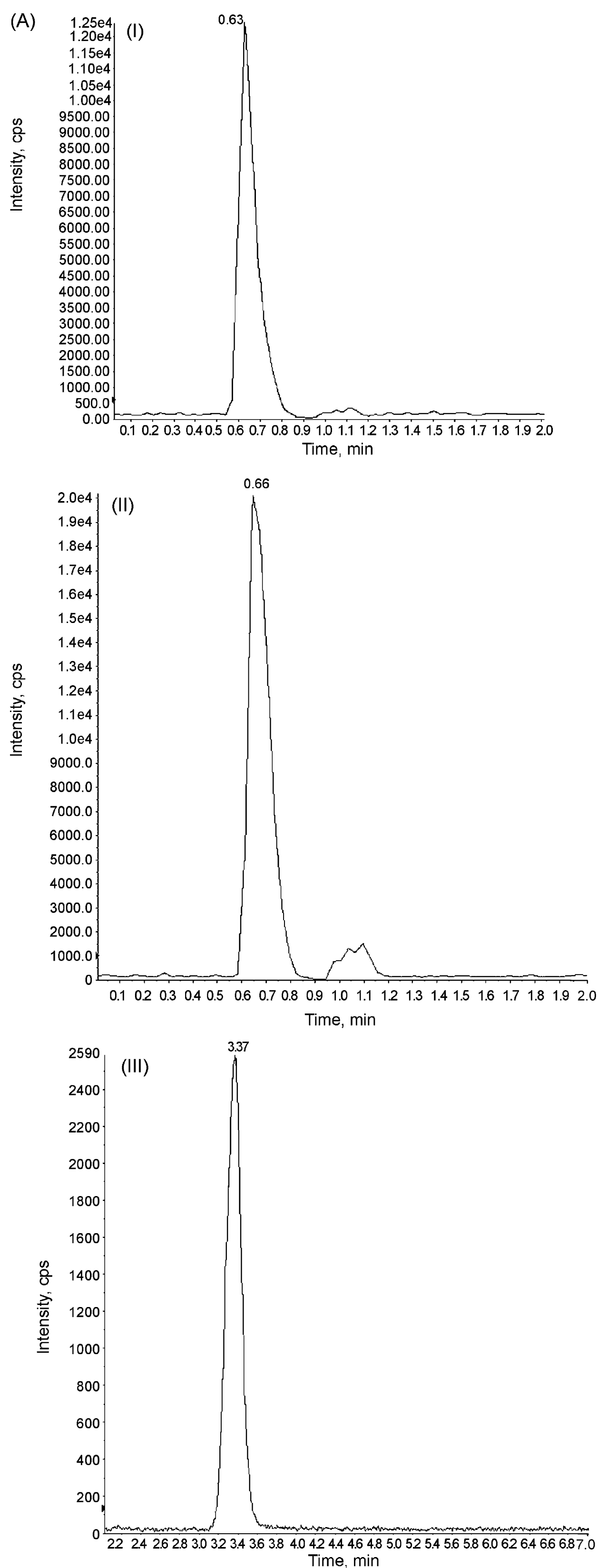

Fig. 3. Representative extracted-ion SRM chromatograms of (A) blank plasma spiked with nebivolol, valsartan and tamsulosin; (B) plasma sample from forehand vein at 2 h after an oral dose of FDC of nebivolol and valsartan to the human subjects. Peak I: nebivolol; Peak II: valsartan; Peak III: tamsulosin (internal standard).

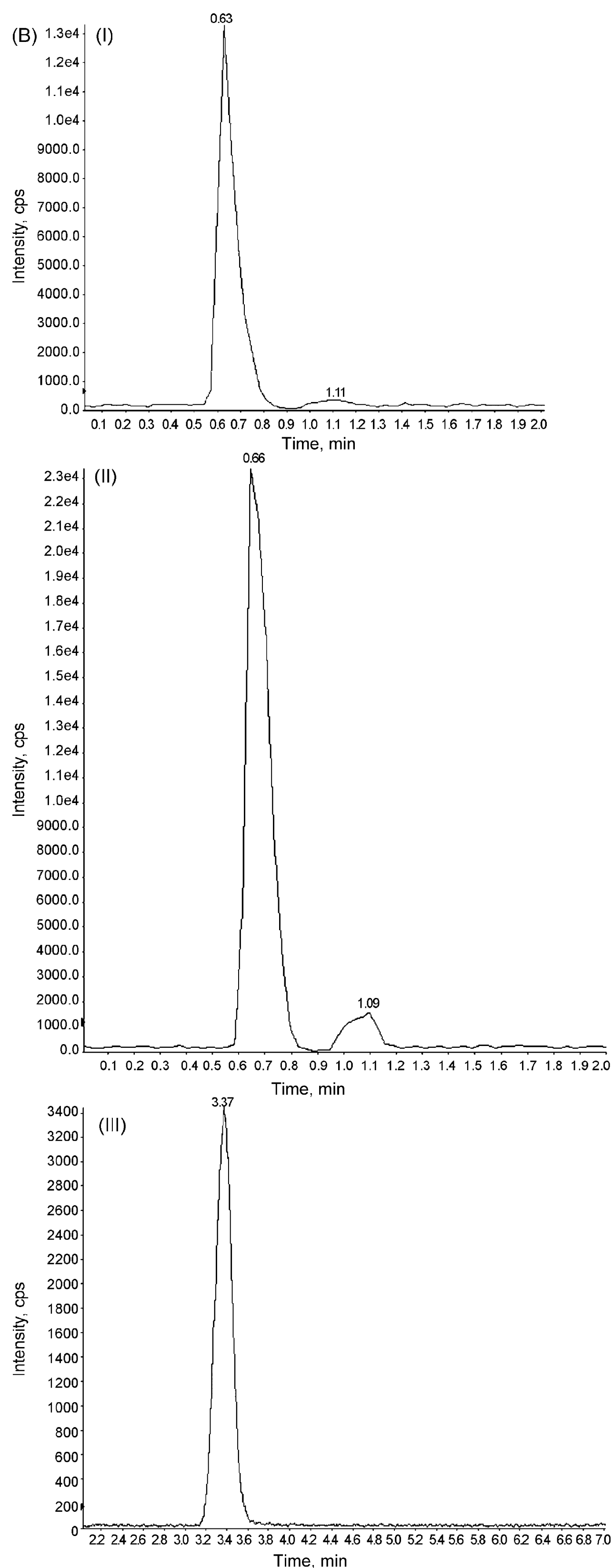

Fig. 3. (*Continued*).

preferred with turbo electrospray ionization (ESI) to quantify the nebivolol and valsartan in human plasma due to its lower levels of background noises. Parameters involving turbo spray needle temperature, heater temperature, flow rate of nebulizing gas and curtain gas were optimized to obtain the protonated molecules for nebivolol, IS and deprotonated molecules of valsartan. The collision energy was optimized to achieve maximum response of the fragment ion peak. SRM mode was used for the detection of nebivolol, valsartan and IS with a dwell time of 100 ms per transition.

3.2. Specificity

The specificity of the method was investigated by comparing chromatograms of 12 different sources of human plasma. The positive product ion mass spectra of the molecular ions of nebivolol, IS and negative product ion mass spectra of the molecular ions of valsartan are shown in Fig. 2. The most intensive product ion was observed at *m/z* 150.9 for nebivolol; *m/z* 179.0 for valsartan and *m/z* 228.1 for IS. The representative chromatograms are shown in Fig. 3, indicating no interferences from endogenous substances in plasma with the analytes and IS. And the nebivolol, valsartan and IS exhibited retention times of ca. 0.63, 0.66 and 3.37 min, respectively. By monitoring the precursor-to-product ion transitions *m/z* 406.1 → *m/z* 150.9; *m/z* 434.2 → *m/z* 179.0 and *m/z* 409.4 → *m/z* 228.1 for nebivolol, valsartan and IS, respectively in the SRM mode, a highly sensitive assay for FDC of nebivolol and valsartan was developed.

3.3. Matrix effect

The ion suppression caused by the plasma matrix was evaluated in this prescribed method. The matrix effect of the method was considerably reduced and suppressed by utilizing the API source and by eliminating a number of endogenous components from plasma extracts during sample preparation. The matrix effect was determined with blank plasma from 12 different sources were spiked with low and high concentrations of 0.025, 40.0 ng/ml for nebivolol and 2.5, 1500.0 ng/ml for valsartan, respectively. No matrix effect and interferences from endogenous compounds were detected in the 12 different sources of human plasma. The relative standard deviation (R.S.D.) value is 4.0% that indicates the reproducibility of peak areas for the 12 extracted aliquots from the blank plasma spiked with low and high QC concentration of nebivolol and valsartan. It also indicates that the extracts were “clean” with no co-eluting “unseen” components interfering with the ionization of the analytes.

3.4. Linearity and sensitivity

The linearity of each calibration curves was determined by plotting the peak area ratio (y) of analytes to IS versus the nominal concentration (x) of nebivolol and valsartan. The calibration curves were obtained by weighted ($1/x$) linear regression analysis. To evaluate the linearity of the LC–MS–MS method, plasma calibration curves were determined in triplicate on three separate days. Good linearity was observed over the concentration range of 0.01–50.0 ng/ml for nebivolol and 1.0–2000.0 ng/ml for valsartan on each calibration. And the correlation co-efficient was 0.9927 and 0.9963 for nebivolol and valsartan, respectively. No significant changes in the values of slope, intercept and correlation co-efficient on both inter- and intra-day calibrations. The lower limit of quantitation (LLOQ) for nebivolol and valsartan were found to be 0.01 and 1.0 ng/ml, respectively in human plasma. The % relative error (R.E.) and % co-efficient of variation (CV) were found to be less than 15.0%, which are sufficient for pharmacokinetic study of nebivolol and valsartan, respectively in human subjects.

3.5. Accuracy and precision

The accuracy was determined by calculating the percentage deviation observed in the analysis of QC samples and expressed in the relative error. The intra- and inter-run precision was expressed as the R.S.D. As shown in Table 2, for each QC level of nebivolol and valsartan, both the intra- and inter-run precision was less than 5.0%, and also the accuracy was within 5.0%, indicating the acceptable accuracy and precision of the method developed.

3.6. Extraction recovery

The extraction recovery of nebivolol and valsartan from human plasma was determined by comparing peak areas from

Table 2
Accuracy and precision of intra- and inter-run analysis for the determination of nebivolol and valsartan in human plasma ($n = 3$ days, six replicates per day)

Added C[a]	Found C[a] (ng/ml), mean ± S.D.	Intra-run R.S.D.[b] (%)	Inter-run R.S.D.[b] (%)	Mean recovery (%)	R.E.[c] (%)
Nebivolol					
0.025	0.025 ± 0.001	2.7	2.0	102.1	1.1
5.0	5.2 ± 0.2	4.2	3.2	103.7	3.7
40.0	40.2 ± 1.8	4.5	3.6	100.5	0.5
Valsartan					
2.5	2.5 ± 0.1	3.2	4.2	101.7	1.7
250.0	252.7 ± 4.1	1.6	2.9	101.1	1.1
1500.0	1555.3 ± 39.1	2.5	4.8	103.7	3.7

[a] Concentration.
[b] Relative standard deviation.
[c] Relative error.

Table 3
Stability data for nebivolol and valsartan in human plasma under various storage conditions ($n = 3$ days, triplicates per day)

Storage conditions	Time	Fresh sample, mean (%) ± S.D.[a]	Stored sample, mean (%) ± S.D.[a]	Deviation (%)	R.S.D.[b] ($n = 3$)
Nebivolol					
4 °C	24 h	100.1 ± 3.2	98.9 ± 2.6	1.1	3.2
Room temperature	24 h	100.2 ± 1.7	101.6 ± 4.2	−1.3	1.7
−20 °C	45 days	103.2 ± 2.2	98.5 ± 2.4	4.6	2.1
24 h; −20 °C	4 cycles	101.2 ± 3.4	99.2 ± 2.6	2.0	3.4
Valsartan					
4 °C	24 h	100.1 ± 3.1	98.3 ± 2.7	1.9	3.1
Room temperature	24 h	97.2 ± 1.7	101.3 ± 4.6	−4.1	1.7
−20 °C	45 days	102.2 ± 1.5	97.6 ± 0.9	4.6	1.4
24 h; −20 °C	4 cycles	99.9 ± 2.9	97.9 ± 2.6	2.0	2.9

[a] Standard deviation.
[b] Relative standard deviation.

plasma samples spiked before extraction with those from plasma samples extracted and spiked after extraction. The results showed that the extraction recoveries were 84.6 ± 4.2, 86.5 ± 2.2, 88.5 ± 3.3% and 86.9 ± 4.7, 83.6 ± 3.7, 84.7 ± 4.0% from human plasma at concentrations of 0.025, 5.0, 40.0 ng/ml and 2.5, 250.0, 1500.0 ng/ml, respectively and the IS was found to be 80.7 ± 4.3% at concentration of 40.0 ng/ml.

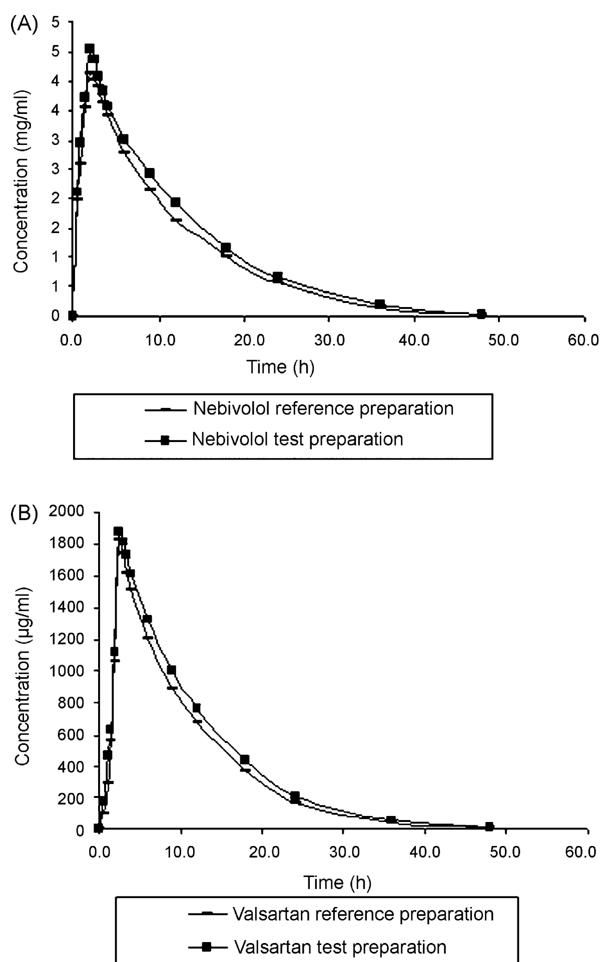

Fig. 4. Mean plasma concentration–time profiles for (A) nebivolol and (B) valsartan after oral administration of test preparation (FDC containing nebivolol 5 mg and vatsartan 80 mg) and reference preparation (Nebicard-5 and Valent-80) to a healthy human volunteer.

Table 4
Pharmacokinetic parameters for a single dose of nebivolol (5 mg) tablet after oral administration to 12 healthy human volunteers

Pharmacokinetic parameter	Reference		Test	
	Mean	±S.D.	Mean	±S.D.
C_{max} (ng/ml)	4.1	0.3	4.6	0.3
T_{max} (h)	2.1	0.3	1.9	0.3
AUC_{0-t} (ng/ml h)	24.7	3.7	25.1	3.9
$AUC_{0-\alpha}$ (ng/ml h)	27.2	3.4	29.4	3.8
$t_{1/2}$ (h)	12.2	3.0	12.8	2.3
K_e (1/h)	0.06	0.003	0.05	0.01

3.7. Stability

The QC plasma samples were stable at 4 °C for 24 h, short-term, long-term stability and freeze-thaw stability studies; the results were showed no significant loss of analytes (Table 3). The stock solutions were stable for at least 30 days and the difference between the assays results were less than 5.0% for both the analytes and IS. The post-preparative samples were stable at room temperature for at least 24 h including the residence time in the autosampler. The final stability test was demonstrated on plasma and mobile phase. No significant deterioration of the analytes was observed under any of these conditions and the mean recoveries were 98.5–104.4 and 97.9–103.5% ($n = 6$) for nebivolol and valsartan, respectively.

Table 5
Pharmacokinetic parameters for a single dose of valsartan (80 mg) tablet after oral administration to 12 healthy human volunteers

Pharmacokinetic parameters	Reference		Test	
	Mean	±S.D.	Mean	±S.D.
C_{max} (µg/ml)	1827.3	54.3	1881.6	32.5
T_{max} (h)	2.4	0.3	2.2	0.2
AUC_{0-t} (µg/ml h)	15,119.2	1223.4	15,954.3	1057.2
$AUC_{0-\alpha}$ (µg/ml h)	60,274.5	2395.7	61,243.6	1877.9
$t_{1/2}$ (h)	7.5	1.0	7.8	0.5
K_e (1/h)	0.09	0.02	0.1	0.01

3.8. Application

This method was successfully applied to the pharmacokinetic study of FDC of nebivolol and valsartan in human plasma. The mean (±S.D.) plasma concentration–time profile for a healthy volunteer after an oral administration of nebivolol and valsartan at the single dose of 5 mg and 80 mg, respectively, is shown in Fig. 4. The values of the main pharmacokinetic parameters are shown in Tables 4 and 5. Pharmacokinetic parameters (T_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\alpha}$, $t_{1/2}$ and K_e) were similar between the reference and test products and the AUC agreed with those published pharmacokinetic studies [5,10].

4. Conclusions

A rapid, sensitive and accurate liquid chromatography with atmospheric pressure ionization-tandem mass spectrometry (LC–API-MS–MS) method was developed for the determination of nebivolol and valsartan in human plasma. The method offers high sensitivity with a low limit of quantitation of 0.01 ng/ml and 1.0 ng/ml, wide linearity, and specificity without interferences from endogenous substances and also in low sample volume. The simplicity of sample preparation facilitates its application in the pharmacokinetic study of FDC of nebivolol and valsartan products. In addition, this high sensitivity method can be used for therapeutic drug monitoring and drug–drug interaction studies in human subjects.

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