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Research Article

Development and validation of a fast and simple HPLC method for the simultaneous determination of bisoprolol and enalapril in dosage form

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Abstract

Aim. We aimed to develop and validate fast, simple, accurate, robust and rugged chromatographic method for simultaneous determination of bisoprolol fumarate and enalaril maleate in solid pharmaceutical dosage form, with using chaotropic strong chaotropic perchlorate anions in composition of mobile phase.

Materials and methods. Fast simple HPLC method for simultaneous determination of bisoprolol and enalapril in solid pharmaceutical dosage forms was developed, with perfect peak symmetries eluting at 4.7 and 5.2 miutes, with mobile phase composed of methanol and diluted perchloric acid pumped with 1ml/min on Zorbax Rx C8 250x4.6mm, 5um column thermostated at 42 °C, and monitored UV signal at 214nm. Mobile phase was composed of 55%methanol and 45% perchloric acid (0.07%v/v).

Results. Usage of presence of perchloric anions showed very useful role in peak shape and chromatogram view, due to distinguished chaotropic characteristics of perchlorate anions on molecules containing nitrogen atoms in molecular structures of analytes, in acidic pH environment. Linearity was examined and proven at different concentration levels in the range of working concentration of bisoprolol (20–200 ug/ml) and enalapril (20–200 ug/ml). The methods achieved very good validation parameters, with determined LOQ about 0.032 mg/ml and LOD about 0.003 mg/ml for bisoprolol, and LOQ about 0.045 mg/ml and LOD 0.005 mg/ml for enalapril. The high value of recoveries obtained for bisoprolol and enalapril indicates that the proposed method was found to be accurate.

Conclusion. A new fast and simple, but selective, accurate, precise and robust HPLC method for simultaneous determination of bisoprolol and enalapril in tablets was developed and many possible variations of the same were suggested. The developed method for the simultaneous quantification of bisoprolol and enalapril from solid dosage formulations offers simplicity essential for quality control of a large number of samples in short time intervals, which is necessary for routine analysis.

Keywords

Bisoprolol, Enalapril, HPLC-UV, Method Development, Validation, Tablets

Introduction

Hypertension is the most common noncommunicable disease, which is accompanied by high mortality among persons of working age and their disability from cardiovascular and cerebrovascular diseases. According to the relevant protocols for the treatment of hypertension are often used antihypertensive drugs of main classes - first-line drugs, which when used in equivalent doses contribute to the reduction of blood pressure and significantly reduce the risk of cardiovascular complications. Quite often, doctors prescribe two/three medicines at a time, which creates some inconvenience for patients and increases the burden on their liver. Therefore, the creation of fixed combinations of API (active pharmaceutical ingredients) antihypertensive action in the form of solid dosage forms is anr task of modern pharmacy.

Bisoprolol is a synthetic, beta1-selective (cardioselective) adrenoceptor blocking agent without significant membrane stabilizing activity or intrinsic sympathomimetic activity in its therapeutic dosage range. The chemical name of bisoprolol fumarate is 1-(propan-2-ylamino)-3-[4-(2-propan-2-yloxyethoxymethyl) phenoxy]propan-2-ol (Fig. 1). The most prominent effect of bisoprolol fumarate is the negative chronotropic effect, resulting in a reduction in resting and exercise heart rate. There is a fall in resting and exercise cardiac output with little observed change in stroke volume, and only a small increase in right atrial pressure, or pulmonary capillary wedge pressure at rest or during exercise.

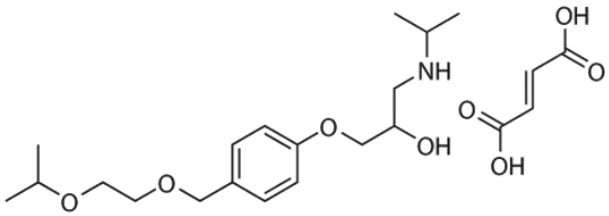

Figure 1. Chemical structure of bisoprolol fumarate.

The State Pharmacopoeia of Ukraine (SPhU) (The State Pharmacopeia of Ukraine 2015) does not have a monograph on the substance of bisoprolol fumarate or on ready medical form. However, the United States Pharmacopoeia regulates the definition of bisoprolol fumarate in substances and tablets. For identification, TLC is proposed (mobile phase – a mixture of dichloromethane, methanol and ammonia solution (70:10:0.8). For the quantitative determination of bisoprolol fumarate in tablets – HPLC/UV. Chromatographic conditions for the determination of drug of Bisoprolol Fumarate, tablets are given in the monograph of the United States Pharmacopoeia, where chromatographic column of L7 category and mobile phase consisting of three components: heptafluorobutyric acid, diethylamine, formate acid are used. Solvent – a mixture of water and acetonitrile (65:35), mobile phase rate – 1 ml/min, detection of wavelength – 273 nm. The proposed method of the United States Pharmacopoeia requires long sample preparation. The European Pharmacopoeia (European Pharmacopoeia 2016) has a monograph on the substance of bisoprolol fumarate. Identification of bisoprolol fumarate EPh regulates to perform the absorption spectrophotometry in the infrared region and the quantitative determination – acidimetry non-aqueous titration.

Enalapril is an angiotensin-converting enzyme (ACE) inhibitor widely used in the therapy of hypertension and heart failure. Enalapril is associated with a low rate of transient serum aminotransferase elevations and has been linked to rare instances of acute liver injury . Chemical name of enapalril is (2S)-1-[(2S)-2-[[(1S)- 1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]pyrrolidine-2-carboxylic acid (Fig. 2).

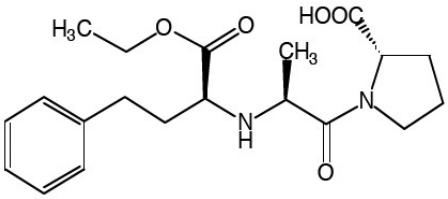

Figure 2. Chemical structure of enalapril.

The State Pharmacopoeia of Ukraine (SPhU) (The State Pharmacopeia of Ukraine 2015) has the monograph on the substance of enalapril maleate and on enalapril tablets. For identification of the substance of enalapril maleate, the SPhU offers the method of absorption spectrophotometry in the infrared region, quantitative determination – alkalimetry potentiometric titration. For identification of enalapril in tablets, the SPhU proposes TLC (mobile phase – a mixture of acetic acid of ice P, water P, butanol P (15:25:60). For the quantitative determination of enalapril in tablets – HPLC/UV (mobile phase – a mixture of acetonitrile P and solvent (40:60), solvent – potassium dihydrogen phosphate solution P, mobile phase rate – 1.0 ml/min, detecting by wavelength at 215 nm). The United States Pharmacopeia regulates the determination of enalapril maleate in substances and tablets. For the identification of enalapril maleate in the substance, the method of absorption spectrophotometry in the infrared region and HPLC/UV, for the quantitative determination – HPLC/UV is proposed. For the identification and quantification of enalapril in tablets, the United States Pharmacopeia offers HPLC/UV. According to this monograph, the following chromatographic conditions are used: chromatographic column of category L1 (bonded reversed phase C18) with size 4.6 mm x 250 mm; mobile phase – a mixture of buffer solution (solution of sodium dihydrogen phosphate with the addition of phosphoric acid to pH2.2): acetonitrile P (75:25); wavelength – 215 nm, flow rate – 2.0 ml/min. The European Pharmacopeia (European Pharmacopoeia 2016) suggests the method of absorbing spectrophotometry in the infrared region for the identification of substance enalapril maleate, quantitative determination – alkalimetry potentiometric titration.

After summarizing all previously published methods, our aim was to create a fast, simple and efficient chromatographic method for the simultaneous determination of bisoprolol and enalapril in dosage form.

Aim of work

We aimed to develop and validate fast, simple, accurate, robust and rugged chromatographic method for simultaneous determination of bisoprolol fumarate and enalaril maleate in solid pharmaceutical dosage form, with using chaotropic strong chaotropic perchlorate anions in composition of mobile phase. It was well documented that strong chaotropic anions significantly contributes to increasing of retentions of N-containing molecules in acidic pH solutions, with improving their peak symmetries, according to Kazakievic 2011.

Materials and methods

The methanol used in experiments was HPLC gradient grade and Perchloric acid 72% and 85% o-Phosphoric acid were of Ph.Eur.Reagent grade and purchased from Merck Darmstdat, Germany, while the deionized water was prepared to conductivity of 0.055 uS. Analytical Balance Mettler Toledo MPC227, pH–metter Metrohm 827, demineralized water from TKA Micro system, with final conductivity less than 0.05µS/cm. IKA orbital shaker KS4000i was used for sample agitation. The nylon and regenerated cellulose RC 0.45um syringe filters were purchased from Agilent Technologies.

Dionex Ultimate 3000 UHPLC system controlled by Chromeleon version 6,80, composed of quaternary LPG pump ultimate 3000, autosampler ultimate 3000, ultimate 3000 column compartment, four channel UV-Vis detector ultimate 3000 RS. Shimadzu Nexera XR UPLC system with LPG Quaternary Pump LC-20AD with degasser DGU-20A5R, Autosempler SIL-20AC, PDA detector M20-A, Column Oven and Controller CBM-20A controlled by Lab Solutions version 5,97. The used column Zorbax Rx-C8 250×4,6mm, 5um, purchased from Agilent Technologies, USA. The standard substances of Bisoprolol Fumarate and Enalapril Maleate were purchased from LGC standards.

Chromatographic conditions

The optimum mobile phase composition was composed of 55% methanol and 45% 0.07% perchloric acid, pumped with 1.0 ml/min at 42 °C set temperature of column oven, with UV detector set to 214nm wavelength, and injection volume for analysis of 10 microliters. Analyses performed on column Zorbax Rx-C8 250×4.6mm, 5um particles.

Sample preparation

Twelve tablets of each preparation were studied to obtain statistically significant results. The tablets with declared contents of 10mg Bisoprolol Fumarate and 10mg of Enalapril Maleate were purchased from local drug store, pharmacy. The tablets were put in 100ml measuring flasks and dissolved in 50ml 10% v/v methanol solution acidified to 0.1% v/v o-phosphoric acid, ultrasound crushed and treated for 2 minutes and shaked 15min with orbital shaker. After that measuring flasks were filled to mark for 100ml, yielding final concetrations of bisoprolol and enalapril to 0.1mg/ml concentration. Samples were filteret with RC 0.45um syringe filters and injected.

Results and discussion

HPLC method was developed to provide specific procedure for the rapid quality control analysis of bisoprolol fumarate and enalapril maleate. To find the appropriate HPLC conditions for separation of the examined drug, various columns, isocratic and gradient mobile phase systems were tried, and successfully attempts were performed using a C8 chromatographic columns Zorbax Rx C8 (4.6 mm i.d. × 250 mm, 5 µm). Perchloric acid 72% is unique in resulting for enalapril, 0.07% V/V yielding pH about 2.1.Increase of column temperature, reducing flow rate and especialy increasing injection volume, enalapril as analyte quantity on-column of enalapril, improves its peak shape, which is in contrary with all other analytes in chromatography, including other present API bisoprolol . The temperature of 42 °C is enough to maintain peak symmetry of enalapril, which is worsening with reducing peak size (quantity). It can be improved if needed in tablets dissolution tests with increasing the concentration of perchloric acid in mobile phase up to 0.15% V/V, without effects on pH stable column like Zorbax Rx C8.Increasing of temperatute is beneficial for peak symmetry improvement espeacing for enalapril but not above 50 °C for longer column life and more injections. Increasing the concentrarion of perchlorate anions may increase retentions and improve peak symmetries of N-containing molecules due to intensifying chaotropic interactions, especially visible phenomenon with using acetonitrile in mobile phases (Kondratova et al. 2016; Logoyda 2018a, 2018b; Logoyda et al. 2018a, 2018b). We tried using mobile phases such as 50% metanol and 50% of 0.07% (V/V) perchloric acid; 55% metanol and 45% of 0.07% (V/V) perchloric acid; 40% metanol and 10% acetonitrile and 50% of 0.07% (V/V) perchloric acid (Fig. 3). The applicability of the mobile phase concept was tested on chromatographic systems and columns with different performances, and the obtained chromatograms are shown in Figs. 4, 5.

Chromatograms were obtained with satisfactory retention factors and very good peak symmetry of both analyte peaks (tailing factors according to USP of around 1.2–1.4), with resolution better than required (R > 7) (Logoyda 2019a or b?). This was accomplished under the following chromatographic conditions: HPLC column Zorbax Rx C8 (4.6 mm i.d. 250 mm, 5 µm), column

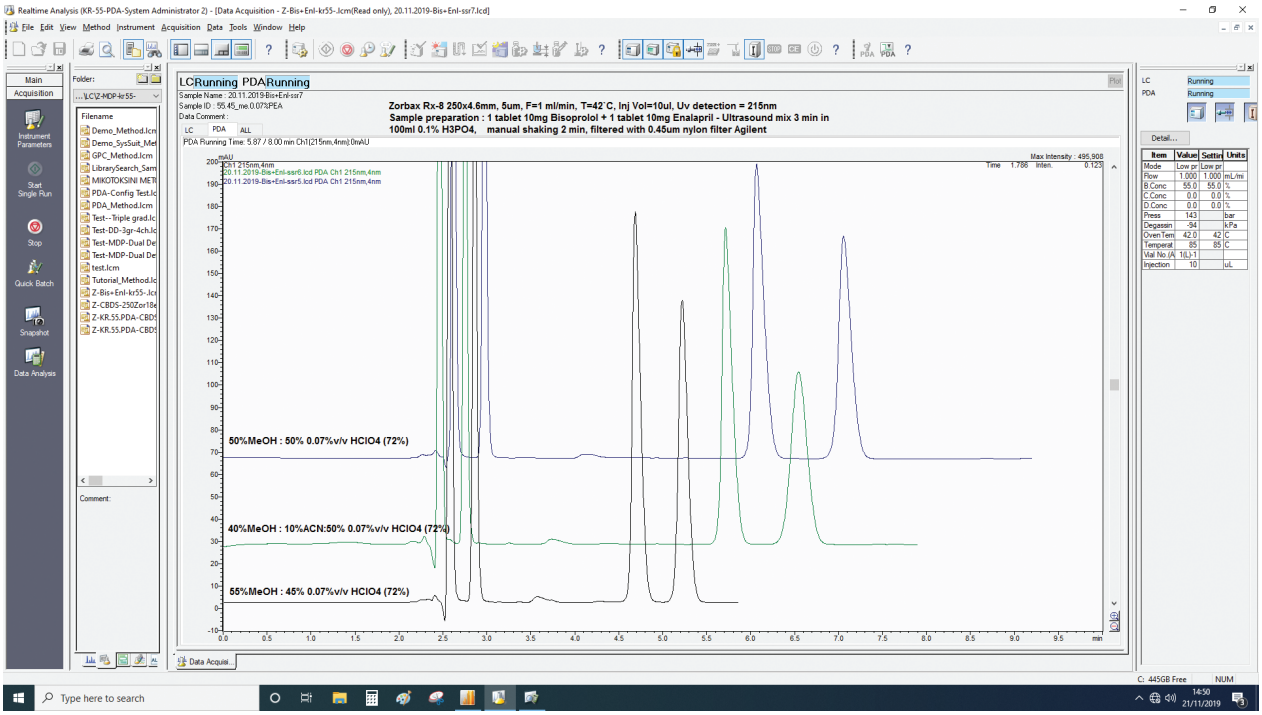

Figure 3. Elution profiles obtained for test samples prepared of bisoprolol+ enalapril tablets (10 + 10) mg using three different mobile phases: **a)** 55% metanol and 45% of 0.07% (V/V) perchloric acid; **b)** 40% metanol and 10% acetonitrile and 50% of 0.07% (V/V) perchloric acid; and **c)** 50% metanol and 50% of 0.07% (V/V) perchloric acid. First peak is bisoprolol and second enalapril. Chromatographic conditions: Shimadzu Nexera XR UPLC system, C-8 column Zorbax Rx (4.6 mm i.d. X 250 mm, 5 µm), flow rate 1.0 mL/min, column temperature 42 °C, injection volume 10 µL.

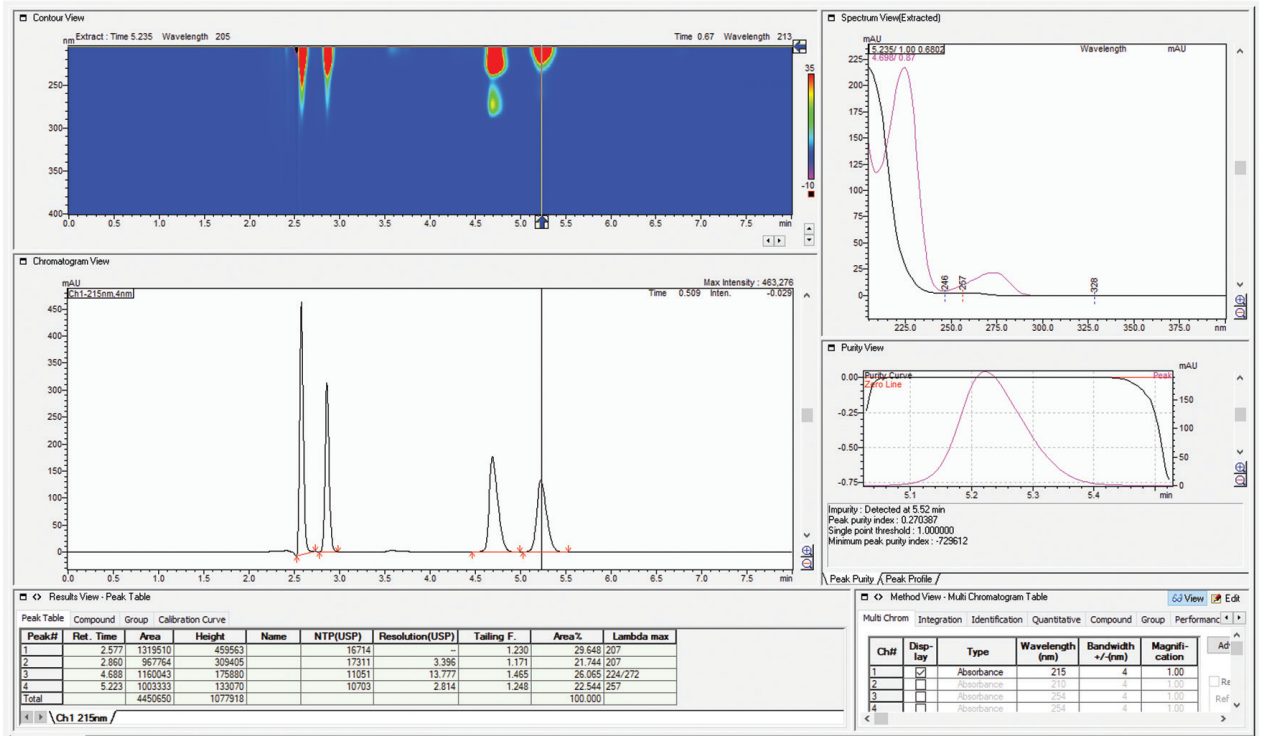

Figure 4. Chromatogram obtained using Shimadzu Nexera XR UPLC system and mobile phase 55% metanol and 45% of 0.07% (V/V) perchloric acid, column Zorbax Rx C8 (4.6 mm i.d. × 250 mm, 5 µm), with 3-D UV contour diagram extracted, analytes UV spectra and peak purity. First peak at about 4.7min is bisoprolol and peak at about 5.2min is enalapril.

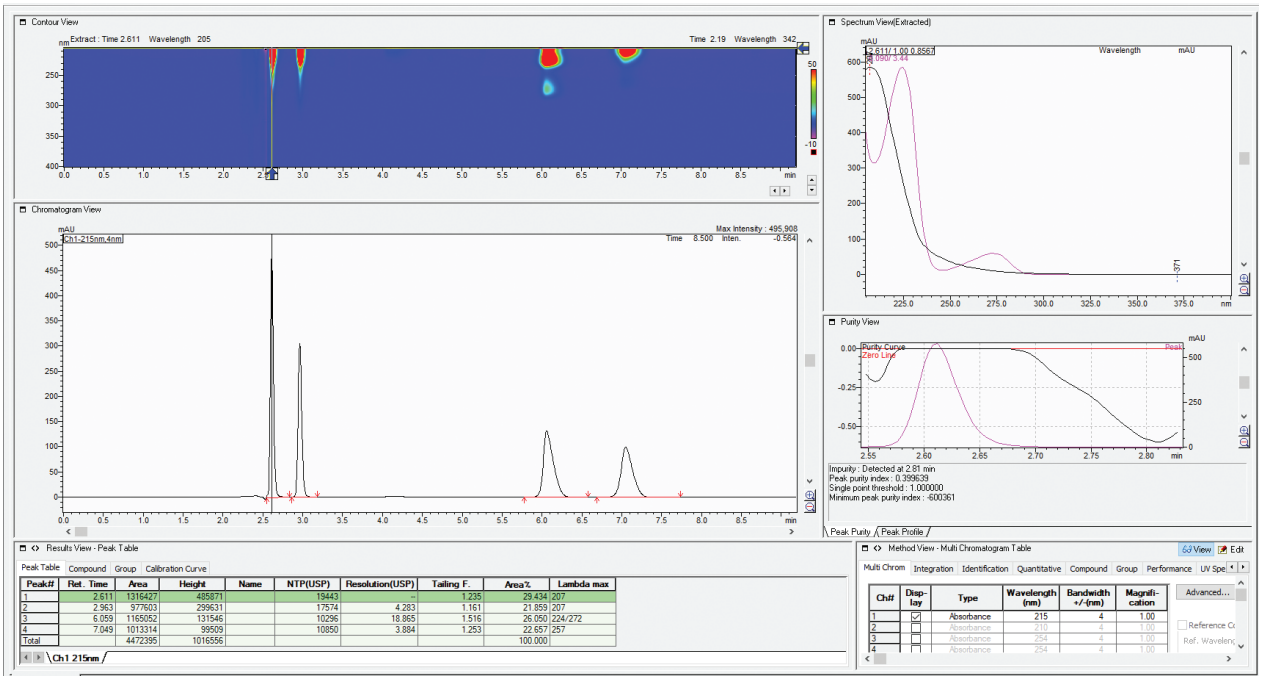

Figure 5. Chromatogram obtained using Shimadzu Nexera XR UPLC system and mobile phase 50% metanol and 40% of 0.07% (V/V) perchloric acid, column Zorbax Rx C8 (4.6 mm i.d. × 250 mm, 5 µm), with 3-D UV contour diagram extracted ,analytes UV spectra and peak purity. First peak at about 6.1min is bisoprolol and peak at about 7.1min is enalapril.

temperature 42 °C, flow rate 1.0 mL/min, mobile phase composed of metanol and 0.07% (V/V) perchloric acid (55:45) and signal monitoring at a wavelength of 215 nm (Fig. 6).

The method was validated according to the ICH guideline for the Validation of analytical procedures Q2((Q1A (R2) 2003, Q2A 1994, Q2B 1996).

Specificity

The selectivity of the method was determined with evaluation of the obtained chromatograms of the blank, placebo solution, test solution and standard solution. The chromatograms showed that there is no interference between the principal peaks of bisoprolol and enalapril with the components of placebo and the used solvent, and also good resolution (Fig. 6).

Linearity

Calibration curve representing the relation between the concentrations of drugs versus the peak area were constructed. In triplicate run from which the linear regression equation was calculated. Chromatogram obtained under linearity study in 11 concentrations levels is presented in Fig. 7 Linearity was examined and proven at different concentration levels in the range of working concentration of bisoprolol (20–200 ug/ml) and enalapril (20–200 ug/ml). The calibration plots of bisoprolol fumarate and enalapril maleate are presented in Fig. 8, 9.

For bisoprolol, linearity regression equation y = 0.1793 x - 0.0103 and an obtained correlation coefficient of R^2 = 0.9999. For enalapril, linearity regression equation y = 0.1817 x + 0.0746 and an obtained correlation coefficient of R^2 = 0.9999. The results show that a phenomenal relationship between peak area and concentration of the drugs in the calibration curves and indicate high sensitivity of the proposed HPLC method. The methods achieved very good validation parameters, with determined LOQ about 0.032 mg/ml and LOD about 0.003 mg/ml for bisoprolol, and LOQ about 0.045 mg/ml and LOD 0.005 mg/ml for enalapril.

Accuracy and precision

System precision is shown in Table 1. Intra-day and inter-day % RSD values lower than 2% clearly assuring that this method was found to be fairly precise and reproducible (Tables 2–5). Regarding accuracy, a known amount of the standard drug was added to the fixed amount of preanalyzed sample solution. % recovery was calculated by comparing the area before and after addition of the standard drug. The high value of recoveries obtained for bisoprolol and enalapril indicates that the proposed method was found to be accurate.

Robustness

The robustness of the developed method was evaluated by small deliberate changes in method parameters such

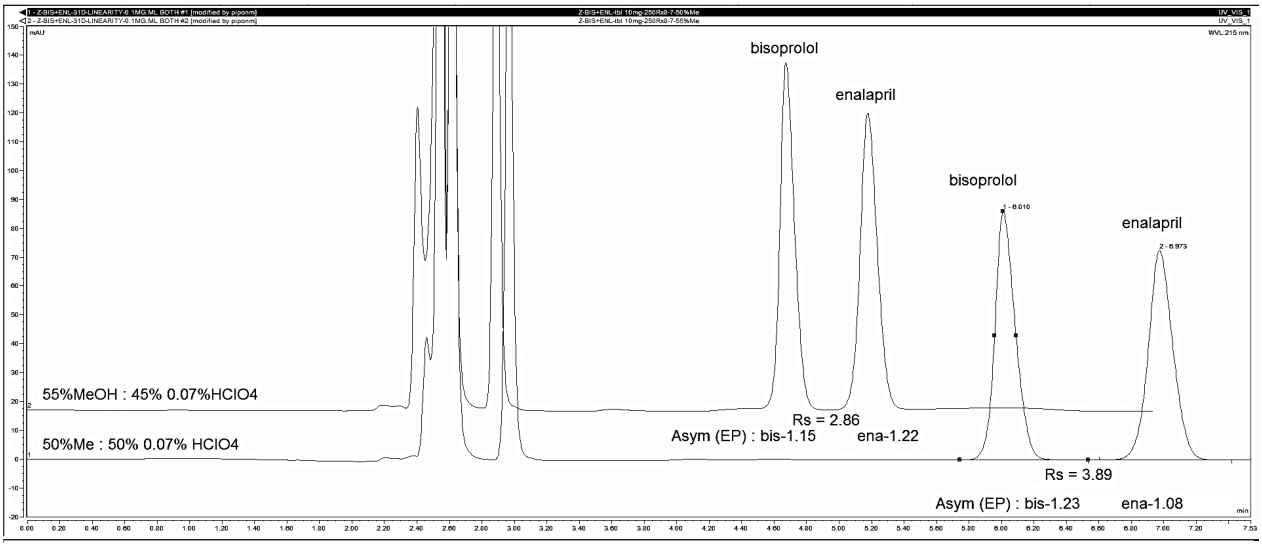

Figure 6. Chromatograms chracteristics obtained using final established optimized validated chromatographic method (upper run), compared with more reteaining with 10% less methanol (lower run), with their system suitabilitiy parameters,worked on Dionex Ultimate 3000 UHPLC system.

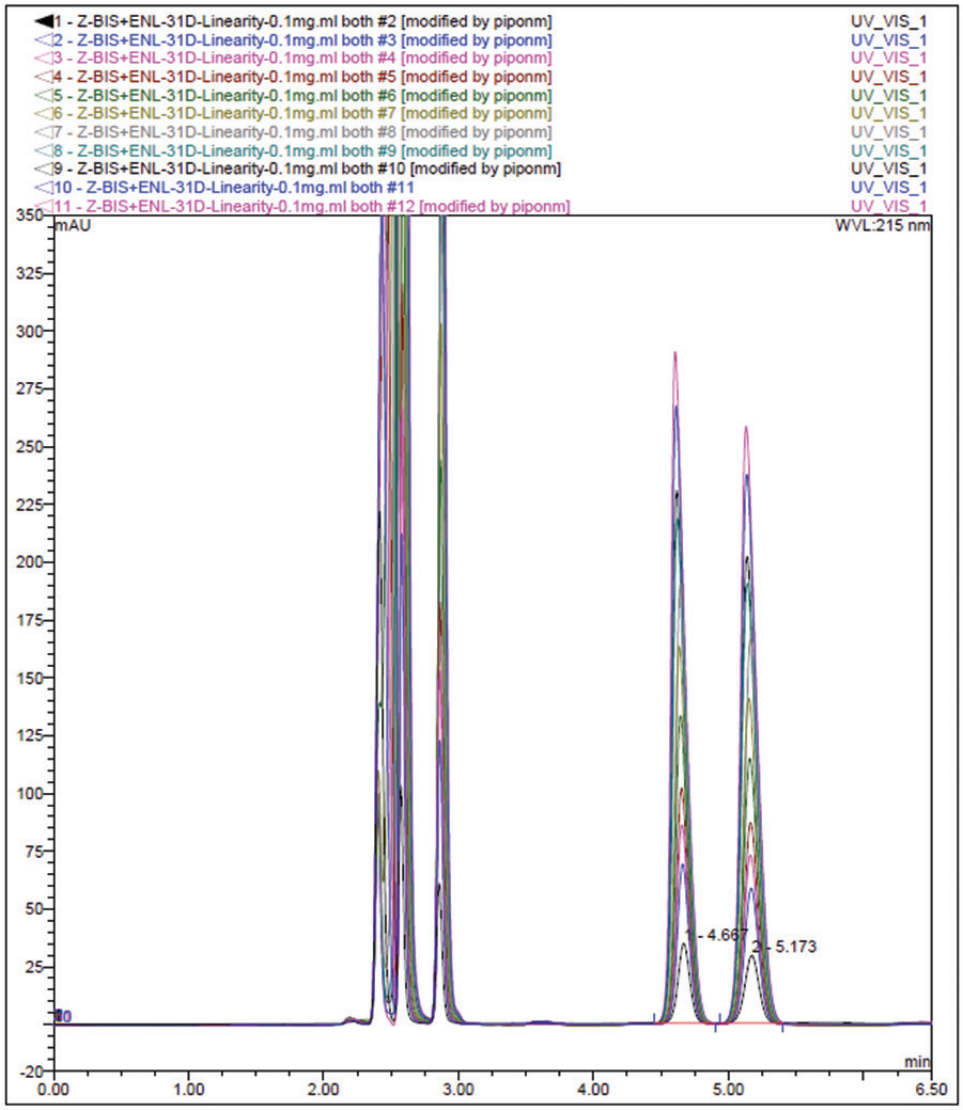

Figure 7. Linearity illustrating chromatograms obtained using final established, optimized and validated chromatographic method, with 55% methanol. First peak is bisoprolol and second enalapril Dionex Ultimate 3000 UHPLC system.

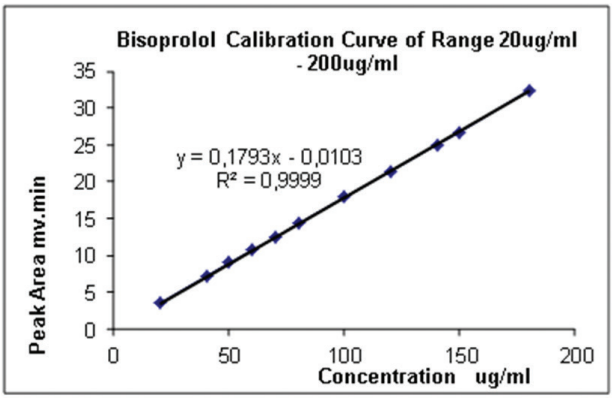

Figure 8. The calibration graph of bisoprolol fumarate.

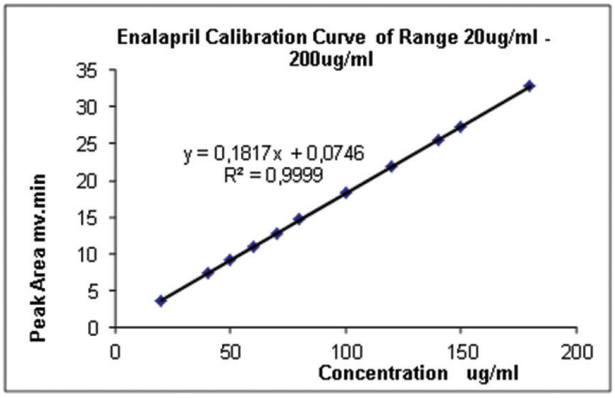

Figure 9. The calibration graph of enalaril maleate.

Table 1. Results of system precision for the HPLC determination of bisoprolol and enalapril.

Nr	Area Bisoprolol	Area Enalapril
1	120184	102817
2	120649	103101
3	120865	102734
4	121012	103327
5	120755	103461
AVR	120693	103088
STD	314.72	314.3
RSD %	0.26	0.30

Table 2. Intra-day and inter-day precision for the HPLC determination of bisoprolol.

Day	Intra-day precision		Inter-day precision	
	Mean	R.S.D %	Mean	R.S.D %
1	99.82	0.311	100.76	0.364
2	100.41	0.647	99.27	0.390
3	100.82	0.336	100.53	0.572

*Each value is represented as a mean±SD of observations, SD: Standard Deviation, RSD: Relative Standard Deviation.

Table 3. Intra-day and inter-day precision for the HPLC determination of enalapril.

Day	Intra-day precision		Inter-day precision	
	Mean	R.S.D %	Mean	R.S.D %
1	99.23	0.353	101.79	0.514
2	101.17	0.719	99.57	0.349
3	100.82	0.376	100.13	0.674

*Each value is represented as a mean±SD of observations, SD: Standard Deviation, RSD: Relative Standard Deviation.

Table 4. Accuracy study for the HPLC determination of bisoprolol.

Model Solutions	The amount of bisoprolol, %		Found,% to predetermined, $Z_i = (Y_i/X_i)\cdot 100\%$
	Predetermined quantity, $X_i=(m_i/m_{rs})$ 100%	Found quantity, $Y_i=(S_i/S_{rs})$ 100%	
M1	69.98	70.03	100.07
M2	80.01	80.12	100.14
M3	89.97	90.02	100.06
M4	95.01	95.09	100.08
M5	100.00	99.95	99.95
M6	104.95	105.04	100.09
M7	110.00	110.08	100,07
M8	120.08	120.17	100.07
M9	130.01	130.11	100.08
Average, *Z*, %			100.07
Standard deviation, S_z, %			0.05
Confidence interval of convergence of results (actual) $\Delta z = t(95\%,8)\cdot S_z = 2.3060\cdot S_z$, %			0.11
Critical value for the convergence of results $\Delta \le max\Delta_{As} = 2.4\%$			Performed (< 2.4)
Systematic error $\delta = \lvert Z - 100 \rvert$, %			0.07
Criterion of significance of systematic error $\delta \le max\ \delta\%$			Performed (< 0.77)
The general conclusion about the technique:			Correct

Table 5. Accuracy study for the HPLC determination of enalapril.

Model Solutions	The amount of enalapril, %		Found,% to predetermined, $Z_i = (Y_i/X_i)\cdot 100\%$
	Predetermined quantity, $X_i=(m_i/m_{rs})$ 100%	Found quantity, $Y_i=(S_i/S_{rs})$ 100%	
M1	70.05	70.12	100.10
M2	80.02	80.19	100.21
M3	90.03	90.17	100.16
M4	95.22	95.41	100.20
M5	100.05	99.89	99.84
M6	104.88	105.01	100.12
M7	110.05	110.32	100.25
M8	120.11	120.15	100.03
M9	130.07	130.14	100.05
Average, *Z*, %			100.11
Standard deviation, S_z, %			0.12
Confidence interval of convergence of results (actual) $\Delta z = t(95\%,8)\cdot S_z = 2,3060\cdot S_z$, %			0.28
Critical value for the convergence of results $\Delta z \le max\Delta_{As} = 2,4\%$			Performed (< 2.4)
Systematic error $\delta = \lvert Z - 100 \rvert$, %			0.11
Criterion of significance of systematic error $\delta \le max\ \delta\%$			Performed (< 0.77)
The general conclusion about the technique:			Correct

Table 6. Results of the study of robustness for the HPLC determination of bisoprolol.

Conditions of analysis	Retention time, min
Standard conditions	4.67
flow rate 1.1 mL/min, (+10%)	4.24
flow rate mL/min, (-10%)	5.13
temperature of column 45 °C	4.34
temperature of column 37 °C	4.98

Table 7. Results of the study of robustness for the HPLC determination of enalapril.

Conditions of analysis	Retention time, min
Standard conditions	5.18
flow rate 1.1 mL/min, (+10%)	4.77
flow rate 0.9 mL/min, (-10%)	5.79
temperature of column 45 °C	4.98
temperature of column 37 °C	5.59

as flow rate (+10%) and temperature of column (± 3%). The % RSD values of robustness which is less than 2% reveals that the proposed method is robust. The results of robustness study results are shown in Tables 6, 7.

Even though the small changes in the conditions did not significantly effect on retention time of bisoprolol and enalapril.

Conclusion

1. A new fast and simple, but selective, accurate, precise and robust HPLC method for simultaneous determination of bisoprolol and enalapril in tablets was developed and many possible variations of the same were suggested. The method is applicable for simoultaneous determination of tablets contents of API and their dissolution testing
2. This proposed method does not use severe conditions for chromatographic columns, avoiding addition of ion-pair reagents, gradient elution and high column temperatures and is characterized with satisfactory results for system suitability and validation parameters.
3. The developed method for the simultaneous quantification of bisoprolol and enalapril from solid dosage formulations offers simplicity essential for quality control of a large number of samples in short time intervals, which is necessary for routine analysis. The concept of mobile phase composition was evaluated and confirmed on different chromatographic systems. The octylsilane (i.e. C8) columns proved to be applicable due to shorter run time of analyses. Furthermore, the developed method showed good results for the tested validation parameters, i.e. it is selective, accurate, linear and precise, and is thus suitable to be used for the simultaneous quantification of bisoprolol and enalapril in combined tablet dosage forms.

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