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Development and Validation of a New Method for Simultaneous Estimation of Ramipril and Carvedilol by HPLC

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Abstract

The main target of this current research work was intended to construct and make affirmation of a facile, economical, very sensitive with high precision and accuracy, reversed-phase high performance liquid chromatographic method for simultaneous estimation of two drugs Ramipril and Carvedilol. The validation parameters were verified based on the standard requirements of International Council for Harmonization (ICH), U.S. Food and Drug Administration (FDA) and United States Pharmacopoeia (USP) by the determination of linearity, accuracy and precision. To develop the method, a C18 column (with a dimension of 250 × 4.6 mm, 5 µ, SUFELCOSIL^{TM} LC-18) was used. The mobile phase was comprised of aqueous KH_2PO_4 buffer solution and acetonitrile at the ratio of 55:45 (V/V) and flow rate was 1 mL per minute. 220 nm wavelength in the ultra-violet region was used to monitor the effluents and the retention times were found at 5.1±0.1 and 8.1±0.1 minutes for Ramipril and Carvedilol, respectively. Percent recovery for both drugs was above 98%, which demonstrated that the accuracy protocol was maintained. The linearity responses of the method for both Ramipril and Carvedilol were higher than 0.995 and the percentage of Relative Standard Deviation (RSD) (precision) for both of these two drugs were lower than the highest permissible limit or less than 2% (according to FDA). Therefore, it is easily perceptible that the corresponding RP-HPLC method was highly accurate, effective, rapid and precise which can offer huge potential for the application of simultaneous assay of Ramipril and Carvedilol in pure forms.

Key words: Ramipril, carvedilol, RP-HPLC, %RSD, accuracy, precision, linearity etc.

Introduction

The International Union of Pure and Applied Chemistry (IUPAC) and widely used chemical name of Ramipril (RP) is (1S,5S,7S)-8-((2S)-2-(((1S)-1-ethoxycarbonyl-3-phenyl-propyl)amino)propanoyl)-8-azabi-cyclo(3.3.0)octane-7-carboxylic acid (Figure 1A). It is a lipophilic substance (Kurade *et al.*, 2009). RP has different functional groups like secondary amine, amide, ester and carboxylic acid. RP which is used to treat serious diseases like severe blood pressure, heart failure and other issues related to blood, is an angiotensin converting enzyme inhibitor. It is converted to active RP in the liver due to the de-esterification of its ester group (Nagarajan *et al.*, 2013). This active RP prevents the conversion of Angiotensin I to Angiotensin II which has the potent vaso-constrictive effects (Raju *et al.*, 2017). The chemical name of Carvedilol (CV) is 1-(9H-carbazol-4-yloxy)-3-[2-(2-methoxyphenoxy) ethylamino] propan-2-ol (Shaalan *et al.*, 2014) (Figure 1B). CV consists of several functional groups such as carbazoles, secondary alcohol and secondary amine (Al-Adl *et al.*, 2017). It is a racemic mixture of S (-) carvedilol which blocks beta (both $ß_1$ and $ß_2$) adrenoceptor and the R (+) Carvedilol blocks the alpha (only α_1) adrenoceptor (Stojanović *et al*., 2005;

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Ahmed *et al.*, 2018; Gannu *et al*., 2007). CV's usage to treat heart diseases, ventricular abnormality and hypertension is widely known (Al-Majed *et al.* 2015; Fadhil *et al.* 2021). CV modulates the electrophysical properties of heart by the interaction with K^+ and Ca^{2+} ion channels. As CV has anti-oxidative properties, so it can easily protect mitochondria from oxidative phosphorylation as well as it can decrease oxidative stress (Prajapati *et al.* 2022; Nadella *et al*. 2018).

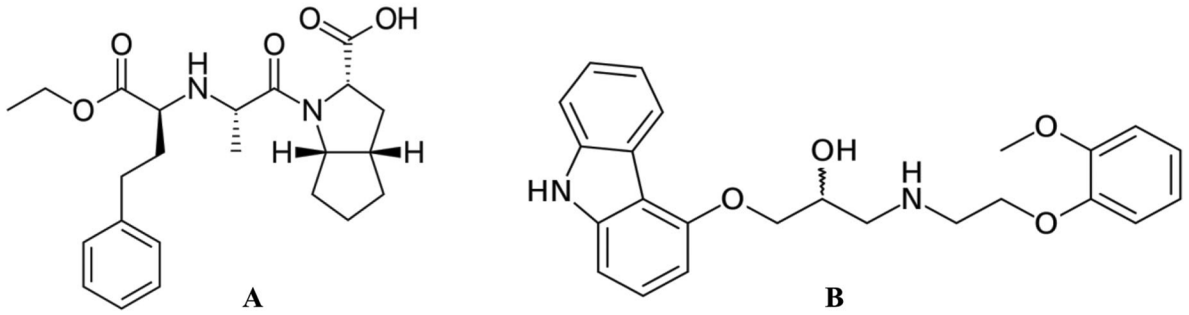

Figure 1. Structures of Ramipril (A) and Carvedilol (B).

A lot of works have been done so far both on RP and CV individually. However, no studies have ever been done to develop and verify a RP-HPLC system for the concurrent assay of RP and CV in either pure or in medicinal forms. The aim of this work is to establish and verify an easy, low cost, less time consuming RP-HPLC method for the quantitation and identification of these two drugs.

Methods and Materials

Chemicals: The pure forms of both RP and CV were bought from Incepta Pharmaceuticals limited, Dhaka, Bangladesh. Both drugs were initially in finely powdered form. The potencies of the both drugs were above 98%. HPLC grade acetonitrile was collected from the local suppliers of the Sigma-Aldrich, Germany. Each of the prepared standard solutions as well as all solvents were filtered through membrane filter of pore size of 0.45 µ by using vacuum pump.

Instrumentations: All analyses were conducted on Prominence HPLC which was equipped with an auto sampler, UV-visible detector, vacuum degasser, dual pumps, column oven. C-18 bonded column (250 × 4.6 mm, 5 µ, SUFELCOSIL™ LC-18) was used to carry out the separation. The mobile phase consisted of KH_2PO_4 buffer and acetonitrile at a ratio of 55:45. The flow rate of the mobile phase was 1.0 mL per minute and the detector was set at 220 nm to monitor the eluate.

Preparation of 0.05M KH_2PO_4 buffer: 6.84 g KH_2PO_4 was weighed and transferred in a 1000 mL volumetric flask and subsequent addition of distilled water in it was done. To fix the pH of the buffer at 3.8, ortho-phosphoric acid solution of concentration 1.0 N was added into it. HPLC grade acetonitrile and the KH_2PO_4 buffer were filtered using a membrane filter.

Preparation of standard solutions of drugs: 5 mg of Carvedilol was dissolved in a mixture of diluents prepared by mixing of acetonitrile and 0.05M KH_2PO_4 buffer in a ratio of 45:55 in a 50 mL volumetric flask. The strength of the prepared solution was 100 µg/ml. In a similar way, 100 µg/mL concentration of Ramipril solution was prepared. From both of the solutions, a mixture of Carvedilol and Ramipril solutions of concentration 50 µg/ml was prepared. By dilution with the diluent a series of concentrations of 16, 18, 20, 22, 24 µg/ml were prepared.

Method validation: The developed method was validated according to ICH or USP protocols (Rahman *et al.*, 2019; Kayesh *et al*., 2017a; Kayesh *et al*., 2017b; Khan *et al.*, 2016). The specificity test

was confirmed as there were no interferences in the chromatograms of both RP and CV. To check the linearity and range, two calibration curves were obtained by plotting the areas under the peaks of both RP and CV against the concentrations of 16, 18, 20, 22, 24 µg/ml. Accuracy, precision (inter-day) of this new method were done under the USP and ICH guidelines. By changing the wavelengths and flow rates, robustness of the method was also confirmed.

Results and Discussion

Method development: To develop an easy, fast and cost effective method for the analysis of RP and CV simultaneously, several polar mobile phases and their mixtures of different ratios were tried. Ultimately, KH_2PO_4 buffer with a concentration of 0.05M and a pH of 3.8 was applied with acetonitrile (55:45). Then high resolution symmetrical shaped peaks with retention times between 5 to 9 minutes were obtained. The theoretical plates for both RP and CV were acceptable.

After developing the mobile phase compositions, the method was validated according to linearity and range, accuracy and precision.

Validation of the newly developed method: Five standard concentrations of 16, 18, 20, 22 and 24 µg/ml solutions of both RP and CV were injected three times for each concentration. By plotting peak areas under the curves against the concentrations of the solutions, two calibration curves were obtained for RP and CV (Figure 2).

Precision: Inter-day precision was carried out for 3 replicates of each concentration of 19, 21 and 23 µg/ml for both RP and CV. The data are given in table 1 and table 2.

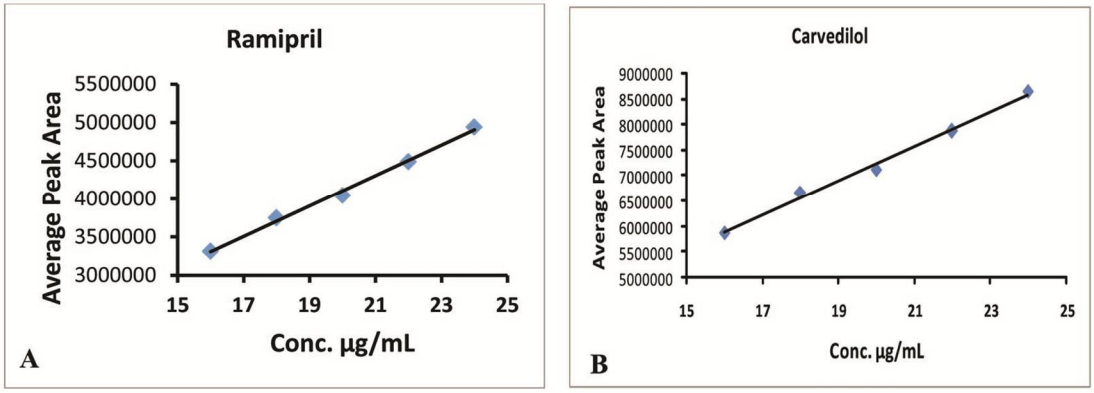

Figure 2. Calibration curve for ramipril (A) and carvedilol (B).

Table 1. The data obtained for method validation parameters.

Parameters	Ramipril	Carvedilol
Linearity equation	y = 199828x + 110457	y = 330770x + 661053
The value of r^2	0.995	0.995
Linearity range	16 -24 µg/ml	16- 24 µg/ml

Accuracy: Accuracy of the standard of RP and CV was determined for 17, 19 and 21 µg/mL concentrations. The data are shown in table 3.

Specificity: There were no interferences in the peaks of both RP and CV which suggest that the method has passed the specificity test. Figure 3 below represents the specificity of the method. The left peak is for RP (Rt 5.145 min) and the right peak is for CV (Rt 8.17 min).

Table 2. The data for inter-day precision (% RSD data for each concentration, n=3).

Concentration in (µg/ml)	Day-1		Day-2		Day-3	
	Ramipril	Carvedilol	Ramipril	Carvedilol	Ramipril	Carvedilol
19	0.983	0.428	2.55	0.684	0.271	0.058
21	0.124	0.283	0.058	0.108	0.250	0.282
23	0.129	0.141	0.101	0.079	0.230	0.042

n= observation number

Table 3. Accuracy data for different concentrations of RP and CV (n=3).

Concentration (µg/ml)	Ramipril	Carvedilol
17	101.4	100.1
19	101.8	100.7
21	101.9	101.4

n= number of determinations.

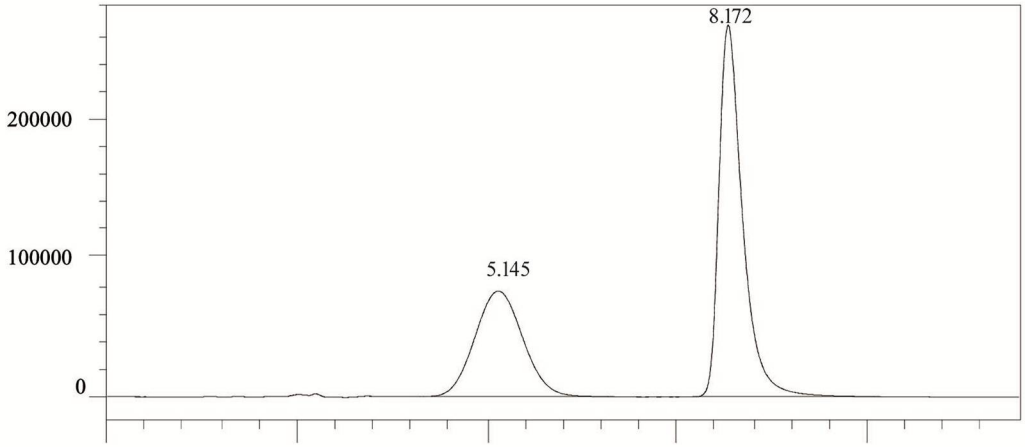

Figure 3. Result of the specificity of the newly proposed method.

Robustness study: The newly developed HPLC method of this study was robust as it showed no significant shifts of the peaks for both RP and CV when the wavelength was changed to 230 and 200 nm. Variation in the rate of flow from 0.8 mL/min to 1.2 mL/min also did shift the peaks on the right side of about 0.1 min. This shift is very insignificant.

Conclusion

Using a C18 column an easy, fast and low cost method was developed with good separation of resolution and the peaks were obtained at 5.145 min to 8.17 min for RP and CV, respectively. The peaks of both RP and CV are symmetrical in shape with the mobile phase constitution of acetonitrile: KH_2PO_4 buffer = 45: 55 (V/V). The method was validated properly by testing the inter-day precision, accuracy, robustness, linearity and range. Therefore, it can be used for the routine analysis of pure drugs RP and CV.

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