ORIGINAL ARTICLE

Development and Validation of Q-Absorbance Ratio UV Spectrophotometric Method for Simultaneous Determination of Bisoprolol Fumarate and Cilnidipine in Marketed Formulation Besicor C5

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Abstract

Introduction: Cardiovascular disorders involve simultaneous administration of more than one drug; hence, numerous combinations, including calcium channel blockers and beta-blockers, are available in the market and are approved by various regulating authorities. Cilnidipine (CLD), a calcium-channel blocker, and Bisoprolol fumarate, a β1-adrenergic receptor blocking agent are such combinations available on the market. This combination is used in the treatment of hypertension and angina pectoris. Therefore, these drugs must be accurately analyzed in various samples, including laboratory mixtures and marketed formulations. **Methods:** Simultaneous estimation of Bisoprolol fumarate and CLD in marketed formulation Basicore C5 was carried out using the UV spectrophotometric method in ethanol. The Q-Absorbance ratio method was selected for estimation. The wavelengths used were 224.5 nm, λmax of Bisoprolol fumarate, and 232 nm, an iso-absorptive point of both the drugs. **Results:** Beer's law was obeyed in the concentration range between 2 and 10 µg/mL for both the drugs. Recovery studies have validated the analysis results as per ICH guidelines. Accuracy was found between 99.3–99.75% and 99.48–100.06% for Bisoprolol fumarate and CLD, respectively. The limit of detection was 0.0943 and 0.0751 µg/mL for Bisoprolol fumarate, while 0.0355 and 0.1141 µg/mL for CLD at 224.5 and 232 nm, respectively. The limit of quantifications was 0.2860 and 0.8983 µg/mL for Bisoprolol fumarate, while 0.1610 and 0.3457 µg/mL for CLD at 224.5 and 232 nm, respectively. **Conclusion:** The method was simple, reproducible, rapid, and precise. Hence, it could analyze laboratory samples and marketed formulations containing these two drugs.

Key words: Bisoprolol fumarate, Cilnidipine, Q-Absorbance ratio method, Simultaneous estimation, UV Spectrophotometric, Validation

INTRODUCTION

Bisoprolol fumarate (BF) is a competitive antagonist at the β1-adrenergic receptor site. It is chemically designed as (RS)-1-{4-[(2-isopropoxy ethoxy) methyl] phenoxy}-3-(isopropyl amino) propan-2-ol.[1,2]. Cilnidipine (CLD) is a calcium channel blocker with vasodilating effect,used as an antihypertensive agent. It is chemically 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3, 5-pyridinedicarboxylic acid 2-methoxyethyl (2E)-3-phenyl-2-propenyl ester.[1,3] The chemical structures of BF and CLD are shown in Figures 1 and 2, respectively. BF and CLD are available in the market as a fixed-dose combination of 5 and 10 mg, respectively, and this combination has utility in various cardiovascular diseases such as hypertension, angina pectoris, and myocardial infarction.[4,5] One of the commercially available combinations is the Besicor C5 tablet by Ajanta Pharma Ltd., India, launched in the Indian

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market in 2017. The present study envisages developing and validating the Q-absorbance UV-spectrophotometric method for simultaneously estimating BF and CLD in their tablet formulation. Determination of BF alone and in combinations with other drugs is reported in the literature by various methods, that is, UV Spectrophotometry,[6-9] HPTLC,[10-12] HPLC,[13-19] and capillary electrophoresis.[20-22] Furthermore, determination of CLD alone or with other drugs by HPTLC,[23-28] HPLC,[29-34] and spectrophotometry[30,35-45] is reported. The literature survey reveals only two reported spectrophotometric methods for simultaneously determining BF and CLDusly.[45,46] One economical and well-used analysis method is UV spectrophotometry, which does not require special instrumentation or tedious separation steps as in chromatographic methods. This UV spectrophotometric method further has several reported methods for simultaneous estimation of drugs, out of which the Q-absorbance method is commonly used and further reported in the literature. A literature survey revealed no Q-absorbance ratio method (Q-method) reported for simultaneous analysis of BF and CLD in pharmaceutical preparations. Hence, developing and validating a new Q-method is required to estimate these drugs in a multi-component mixture simultaneously. The present research reports a simple, precise, and accurate Q-method to determine these two drugs simultaneously. Validation method was done as per the ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents

BF and CLD active pharmaceutical ingredients were supplied as gift samples by Glenmark Pharmaceuticals Ltd, R&D, Taloja, Mumbai, and Alkem Laboratories Ltd, R&D, Mumbai, India, respectively. The certified purity of BF and CLD was 99.05% and 98.97%, respectively. Solvents used were of analytical grade and purchased from Merck (Germany). The solutions used to prepare standard and test solutions were prepared with ethanol.

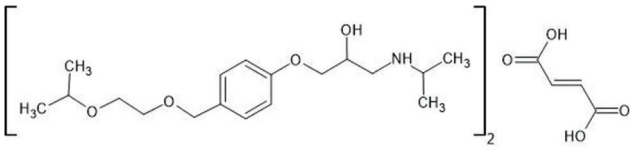

Figure 1: Structure of BF

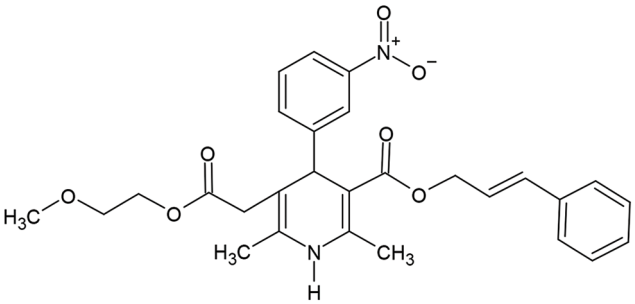

Figure 2: Structure of CLD

Besicor C5 (BF 5 mg and CLD 10 mg) tablets manufactured by Ajanta Pharma Ltd.India were procured from the chemist shop. The solvents were selected based on the solubility of pure drugs and formulation in various solvents such as water, methanol, ethanol, isopropyl alcohol, acetone, and chloroform. The drugs were freely soluble in ethanol. Therefore, ethanol was chosen as a solvent for this study.

Instrument and software

Shimadzu PharmaSpec UV-1700 spectrophotometer (Tokyo, Japan), with preset slit width, quartz cuvette of 1 cm was used for UV-visible measurements of the absorption spectra. Regression and statistical analyses were performed with Microsoft Excel 2010.

Preparation of standard stock solutions

Graduated long neck flask used to prepare the stock solutions, working solutions, and formulation solutions were calibrated before use. 100 mg of BF and CLD were accurately weighed to prepare standard solutions into a 100 mL volumetric flask by dissolving them in ethanol. Solutions were further diluted with the same solvent to obtain substock solution of 100 µg/mL concentration.

Determination of wavelength of maximum absorbance (λmax) and iso-absorptive point

Solutions of 10 µg/mL for both drugs were prepared from a working substock solution, scanned in the range of 200–400 nm against ethanol as blank, and presented in Figures 3 and 4. The overlaying spectrum was also obtained to determine the iso-absorptive point and is presented in Figure 5.

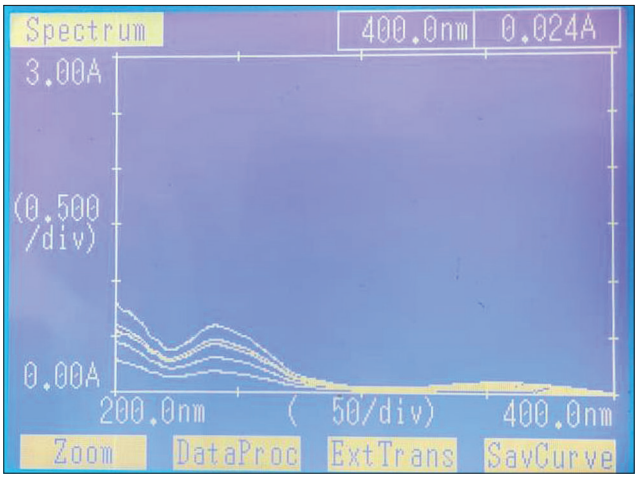

Figure 3: Spectra of different concentrations of BF

Preparation of working solutions from standard substock solution

The working solutions were prepared by diluting standard substock solutions of BF and CLD in different ratios within their concentration linearity ranges. The solutions were carefully diluted to obtain 2–10 µg/mL concentration for both to construct the calibration curves.

Calibration curve (Linearity)

Accurately measured working substock solution of BF (0.2, 0.4, 0.6, 0.8, and 1.0 mL) and working stock solution of CLD (0.2, 0.4, 0.6, 0.8, and 1.0 mL) was transferred to two different series of 10 mL volumetric flask and diluted up to the mark with ethanol. The absorbance of both solutions at their respective λmax and the iso-absorptive point was collected. The calibration curves were drawn with Microsoft Excel by plotting concentration versus absorbance; an average of three determinations was selected as absorbance.

Procedure - Q method

The Q-method involves the absorbance ratio at the iso-absorptive point and λmax of any drug. Iso-absorptive point is the wavelength of equal absorptivity of the two species, where the absorbance ratio at a particular wavelength is a constant irrespective of concentration or path length. At an iso-absorptive point, drug solutions of the same concentration exhibit similar absorbance.[47]

Determination of spectral characteristics of BF and CLD was performed by scanning UV absorbance spectra of each component individually and together with their mixture over a range between 200 and 400 nm against ethanol as a blank [Figure 5]. The absorbance data for solutions were collected from overlay spectra of two drugs; the two wavelengths designated for the progress of the Q-absorbance ratio were 224.5 (λmax of BF) and 232 nm (iso-absorptive point). The absorbance of both drugs was determined, and the absorptivity values were calculated at the two selected wavelengths. These values were a mean of three assessments.

The absorptivity coefficients (Qx and Qy) for BF and CLD were determined. The concentration Cx and Cy of both drugs was calculated using the following equation, respectively;

$$Cx = \frac{Qm - Qy}{Qx - Qy} \times \frac{A1}{ax1}$$

$$Cy = \frac{Qm - Qx}{Qy - Qx} \times \frac{A2}{ay1}$$

here, Absorbance ratio Qm = A2/A1, Qx = ax2/ax1 and Qy = ay2/ay1

where A1 and A2 are the absorbance's of BF and CLD mixture at 224.5 nm and 232 nm;

ax1 and ax2 are absorptivities of BF at 224.5 nm and 232 nm, respectively, and

ay1 and ay2 are absorptivities of CLD at 224.5 nm and 232 nm, respectively.

Application of spectrophotometric methods for marketed formulations besicor C5

Twenty tablets were accurately weighed and powdered in a mortar. Quantity equivalent to one tablet was transferred into a volumetric flask of 100 mL capacity. Powdered drugs were dissolved with vigorous shaking and made up the volume upto 100 mL with ethanol. The solution was filtered through a Whatman filter paper, and residues were rinsed thrice with

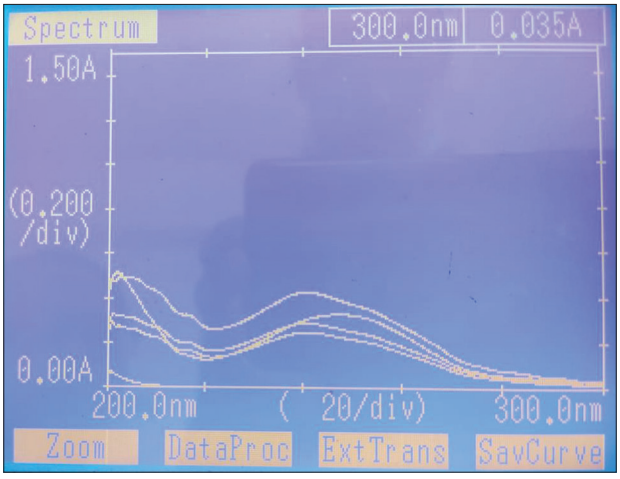

Figure 4: Spectra of different concentrations of CLD

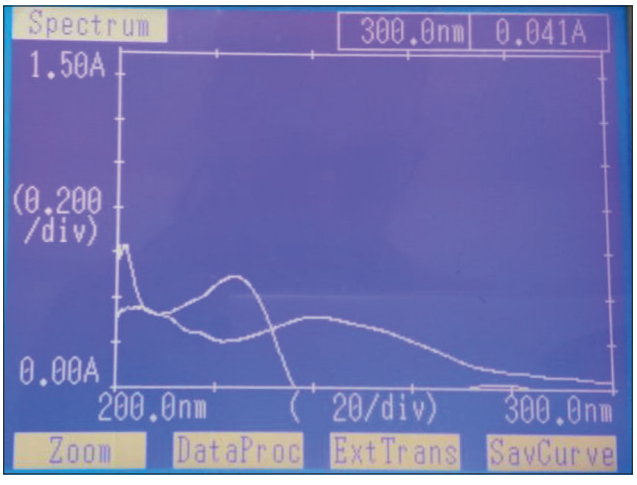

Figure 5: UV scan of BF and CLD showing wavelength of maximum absorbance and iso-absorptive points

ethanol. It was diluted appropriately to obtain the desired concentration and scanned over a range between 200 and 400 nm against ethanol as a blank. The absorbance data for the solution were collected.

Analytical method validation

The developed UV spectrophotometric method was validated according to ICH Q2 (R1) guidelines. The parameters such as linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) were selected for this method validation.

Linearity

Linear regression analysis was performed to assess method linearity. The average of three authentic calibration curves, at 2, 4, 6, 8, and 10 µg/mL concentrations, was used for the same. The linear regression analysis was performed on the selected wavelengths.

Precision and accuracy

Method precision was assessed on two levels intermediate precision and repeatability. The method was tested for these two precision levels based on six determinations of 4 and 8 µg/mL for BF and CLD, respectively, at various time intervals on the same day (intra-day precision) and three dissimilar days (inter-day precision). The standard deviation and %Relative Standard Deviation for the inter-day and intra-day precision were determined. Intermediate precision was performed intra-day and inter-day at 100% of test concentration.

Accuracy (% recovery)

Accuracy was performed using the standard addition method at three levels of 100±20% test concentration, and the percentage recovery from the spiked solution was determined.

LOD and LOQ

As per ICH guidelines, the standard deviation approach determines the LOD and LOQ.LOD and LOQ show the sensitivity of the analytical method, and calculations were done according to the following equation:

$$LOD = 3.3\frac{\sigma}{m}$$

$$LOQ = 10\frac{\sigma}{m}$$

Where σ represents the standard deviation of the y-intercepts of the regression line, and mis the mean slope of the calibration curves.

RESULTS AND DISCUSSION

Method validation

The Q-absorbance method was validated for linearity, accuracy, precision, and LOD and LOQ of analytes in the experimental conditions, and ICH guidelines were followed. The optical regression characteristic is tabulated in Table 1.

Linearity

For constructing calibration curves [Figures 3 and 4], a series of five solutions consisting of 2–10 µg/mL of BF and CLD were analyzed in triplicate. All the experiments maintained optimized conditions, and the regression parameters are summarized in Table 2.Three authentic calibration curves obtained the linear regression equations for the drugs at 224.5 nm [Figure 6] and 232 nm [Figure 7]. Obtained correlation coefficient represents the excellent quality of the calibration curve, as the lower the dispersion of the set of points, the lower the uncertainty of the estimated regression coefficient. As per Beer's Law, linearity was checked and attained in the range of 2–10 µg/mL; selection of the dilution range was based on the ratio of analytes present in the pharmaceutical preparation and the projected concentration in the *in vitro* dissolution studies. The intercepts of the curves were also not significant, representing the excellent linearity in this range of concentrations.

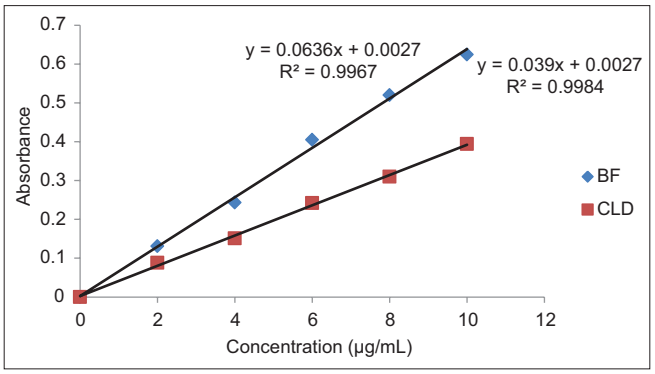

Figure 6: Calibration curve of BF and CLD at 224.5 nm

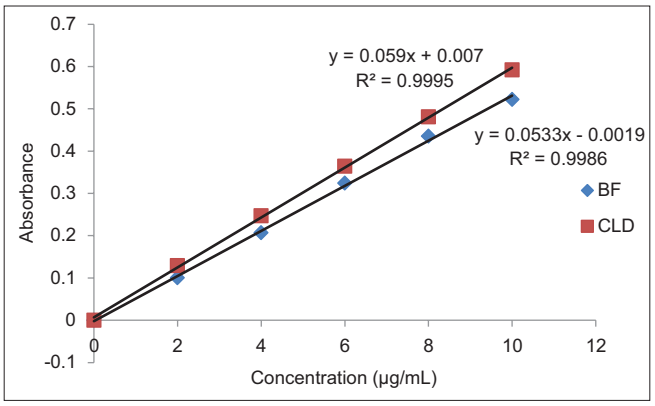

Figure 7: Calibration curve of BF and CLD at 232 nm

Accuracy and precision

Accuracy was proved by the standard addition method and is represented as percent recovery. The proposed method was accurate as percent recoveries were in the range of 99.3 and 99.75% for BF and 99.48 and 100.06% for CLD [Table 3]. Intra-day, inter-day accuracy, and precision were checked using 4 and 8 µg/mL for BF and CLD, respectively. All the analyses were performed in triplicate. For intraday, all the three sets of solutions were analyzed on the same day; for inter-day, all three sets of solutions were analyzed for three consecutive days. Repeatability and intermediate precision were evaluated, and percent relative standard deviation (%RSD) values were calculated. The values of %RSD were found to be less than two, indicating good precision. The precision data are represented in Tables 3 and 4.

LOD and LOQ

The concentration levels of LOD and LOQ were determined by analyzing prepared standard solutions of BF and CLD. These LOD and LOQ were found to be in the concentration range of the solution prepared. The LOD were 0.0943 and 0.0751 µg/mL for BF, while 0.0355 and 0.1141 µg/mL for CLD at 224.5 and 232 nm, respectively. The LOQ was 0.2860 and 0.8983 µg/mL for BF, while 0.1610 and 0.3457 µg/mL

Table 1: Optical regression characteristic and validation parameters

Drug	BF		CLD	
Wavelength (nm)	224.5	232 (Iso-absorptive point)	224.5	232 (Iso-absorptive point)
Linearity range (µg/mL)	2–10	2–10	2–10	2–10
Regression equation	y=0.0636x+0.0027	y=0.039x+0.0027	y=0.0533x−0.0019	y=0.059x+0.007
SD of Y-intercept (*n*=3)	0.0018	0.0012	0.0006	0.0020
Mean of slope (*n*=3)	0.0629	0.0534	0.0387	0.0579
Correlation coefficient (r^2)	0.9967	0.9984	0.9986	0.9995
LOD (µg/mL)	0.0943	0.0751	0.0355	0.1141
LOQ (µg/mL)	0.2860	0.8983	0.1610	0.3457

BF: Bisoprolol Fumarate, CLD: Cilnidipine

Table 2: Calibration points of the standard curve with SD and %RSD

Concentration (µg/mL)	At 224.5 nm				At 232 nm (Iso-absorptive point)			
	BF		CLD		BF		CLD	
	Mean Absorbance SD (*n*=3)	%RSD	Mean Absorbance SD (*n*=3)	%RSD	Mean Absorbance SD (*n*=3)	%RSD	Mean Absorbance SD (*n*=3)	%RSD
2	0.1310±0.002	1.5267	0.0880±0.0015	1.7292	0.0986±0.0015	1.5485	0.1270±0.0020	1.5748
4	0.2413±0.0020	0.8625	0.1500±0.0015	1.0138	0.2053±0.0020	1.0137	0.2440±0.0030	1.2295
6	0.4033±0.0020	0.5161	0.2420±0.001	2.0408	0.3226±0.0015	0.4734	0.3616±0.0025	0.6958
8	0.5176±0.0032	0.6209	0.3150±0.001	1.5625	0.4333±0.0020	0.4803	0.4793±0.0020	0.4342
10	0.6216±0.0025	0.4048	0.3933±0.001	1.3333	0.5190±0.0030	0.5780	0.5886±0.0035	0.5965

BF: Bisoprolol Fumarate, CLD: Cilnidipine

Table 3: Accuracy and precision data by % recovery studies of BF and CLD

Drug	Levels %	Known Conc. taken (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL)	Amount of std drug recovered (µg/mL)	% Recovery±SD (*n*=3)
BF	80	4	3.2	7.2	7.15	99.3012±0.0070
	100		4	8	7.98	99.7535±0.0141
	120		4.8	8.8	8.76	99.5447±0.0092
CLD	80	8	6.4	14.4	14.35	99.6512±0.0183
	100		8	16	16.01	100.0617±0.0254
	120		9.6	17.6	17.51	99.4843±0.0465

BF: Bisoprolol Fumarate, CLD: Cilnidipine

for CLD at 224.5 and 232 nm, respectively. The calculated LOD and LOQ are also represented in Table 1.

Selectivity

The developed method was checked for selectivity by analyzing two different mixture solutions prepared in the laboratory at different ratios; the selections of the concentrations for both the drugs were taken in the linearity range but are above and below the concentration of the marketed formulation, that is, 50% and 150%. Evaluations are done in triplicates. The selectivity results were 98.85–100.95% for BF and 98.57–99.86% for CLD and are presented in Table 5; the results confirm the selectivity of the developed analytical procedures to analyze the various ratios of both drugs. Results of selectivity also rationalize that the method may be utilized to analyze intermediate dissolution samples of marketed formulation, which will be concentrated below the final marketed solution and can be applied for other developing formulations in other ratios. The results suggest that the method could quantitatively analyze both drugs with high accuracy, selectivity, and precision.

Determination of BF and CLD in the formulation

The marketed formulation Besicor C5 (Ajanta Pharma ltd), available in dose ratios of 1:2 (5 mg BF and 10 mg CLD), was analyzed using two different concentrations in the same ratio. The results of the same are presented in Table 6. The standard addition method evaluated the percent recoveries at three different concentrations, 50%, 100%, and 150%. The additions were made to an earlier evaluated tablet solution comprising 4 and 8 µg/mL equivalent of BF and CLD. The assay results [Table 7] of

Table 4: Intermediate precision study

Precision	% Assessment of BF±SD (*n*=3)	%RSD	% Estimation of CLD±SD (*n*=3)	%RSD
Intraday Precision	99.6999±0.463	0.4260	99.4614±0.6456	0.7546
Intermediate Precision	99.7987±0.277	0.5810	99.5535±0.2380	0.6874

BF: Bisoprolol Fumarate, CLD: Cilnidipine

Table 5: Selectivity study

Drug	Levels %	Concentration (µg/mL)	Amount of std drug recovered (µg/mL)	% Recovery±SD (*n*=3)
BF	50	2	2.0193	100.9515±0.0057
	100	4	3.9542	98.8552±0.0024
	150	6	5.9647	99.4139±0.0006
CLD	50	4	3.9428	98.5794±0.0015
	100	8	7.9568	99.4606±0.0009
	150	12	11.9168	99.2506±0.0007

BF: Bisoprolol Fumarate, CLD: Cilnidipine

Table 6: Analysis of pharmaceutical dosage form

Tablet formulation	Label claim (mg)		Amount found		% Assay±SD (*n*=3)	
Besicor C5	BF	CLD	BF	CLD	BF	CLD
	5	10	4.91	9.93	98.2121±0.0680	99.3795±0.0430

BF: Bisoprolol Fumarate, CLD: Cilnidipine

Table 7: Determination of formulation by standard addition method

Drug	Levels %	Conc. taken (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL)	Amount of std drug recovered (µg/mL)	% Recovery±SD (*n*=3)
BF	50		2	6	5.96	99.3349±0.0634
	100	4	4	8	7.89	98.6251±0.0054
	150		6	10	9.92	99.2251±0.0129
CLD	50		4	12	11.93	99.4163±0.0072
	100	8	8	16	16.27	101.6808±0.0521
	150		12	20	19.68	98.4161±0.0003

BF: Bisoprolol Fumarate, CLD: Cilnidipine

the Besicor C5 were concurrent with the actual amount of both analytes. The standard ranges of percent recovery demonstrate the noninterference of formulation excipients in the analysis.

CONCLUSIONS

A reliable Q absorbance ratio method is developed to simultaneously estimate BF and CLD in combined pharmaceutical formulation using UV Spectrophotometry. The proposed method was precise, accurate, and reproducible and had acceptable recovery of the analytes. The excipients and additives commonly present in the combined dosage form showed no interference in drug analysis. Hence, the method can be suitably adapted for regular quality control analysis of the BF and CLD in a mixture or combined pharmaceutical formulation.

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WORK SHOULD ATTRIBUTE TO

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REFERENCES

1. Indian Pharmacopoeia. Indian Pharmacopoeia Commission. Vol. 2. Ghaziabad, India: Ministry of Health and Family Welfare. Government of India; 2018. p. 1392-3, 1616-8.
2. The United States Pharmacopoeia (USP 29). The National Formulary (NF 24). Rockville, USA: United State Pharmacopieal Convection Inc; 2006. p. 292-4.
3. Japanese Pharmacopoeia. Society of Japanese Pharmacopoeia. Tokyo, Cilnidipine: Japanese Pharmacopoeia; 2016. p. 704-5.
4. Available from: https://www.1mg.com/drugs/besicor-c-5-tablet-340787 [Last accessed on 2020 Dec 16].
5. Available from: https://www.apollopharmacy.in/otc/besicor-c-5-tab-10-s [Last accessed on 2020 Dec 16].
6. Hassan SA, Nashat NW, Elghobashy MR, Abbas SS, Moustafa AA. Advanced chemometric methods as powerful tools for impurity profiling of drug substances and drug products: Application on bisoprolol and perindopril binary mixture. Spectrochim Acta A Mol Biomol Spectrosc 2022;267:120576.
7. Mohammed S, Adam M, Shantier S. Development and validation of UV spectrophotometric method for determination of bisoprolol fumarate in bulk and pharmaceutical dosage forms. Mediterr J Chem 2017;6:196-9.
8. Gudruman AD, Murarasu A, Dorneanu V. Spectrophotometric determination of bisoprolol using methyl orange as reagent. Farmacia 2012;60:634-40.
9. El-Didamony AM, Saad MZ, Ramadan GM. Kinetic spectrophotometric determination of β-adrenergic antagonists using alkaline potassium permanganate. Main Group Chem 2012;11:245-58.
10. Jadhav NB, Yadav SS. Analysis of bisoprolol fumarate and amlodipine besylate in tablet dosage form by using HPTLC. Int J Pharm Sci Rev Res 2019;58:75-8.
11. Kakde RB, Kotak VH, Kale DL. Estimation of bisoprolol fumarate in pharmaceutical preparations by HPTLC. Asian J Res Chem 2008;1:70-3.
12. Yadav SS, Rao JR. Simultaneous HPTLC analysis of bisoprolol fumarate andhydrochlorthiazide in pharmaceutical dosage form. Int J Pharm Pharm Sci 2013;5:286-90.
13. Piponski M, Balkanov T, Logoyda L. Development and validation of a fast and simple HPLC method for the simultaneous determination of bisoprolol and enalapril in dosage form. Pharmacia 2021;68:69-77.
14. Beula SJ, Suthakaran R, Bichal PK, Shankar CH, Ghouse S, Suneetha K. A prospective validation of hydrocholorothiazide and bisoprolol fumerate in its pure and bulk dosage forms by using RP-HPLC technique. Asian J. Pharm. Anal 2020;10:1-6.
15. Zaki MM, Abdelwahab NS, Ali AA, Sharkawi SM. Simultaneous determination of bisoprolol fumarate and rosuvastatin calcium in a new combined formulation by validated RP-HPLC. Eur J Chem 2019;10:52-6.
16. Mostafa A, El-Gindy A, Emara S. Simultaneous spectrophotometric estimation of bisoprolol fumarate and hydrochlorothiazide in tablet formulation using partial least-squares, principal component regression multivariate calibrations and RP-HPLC methods. J Anal Pharm Res 2017;4:00124.
17. Raju DS, Vidyadhara S, Rao BV, Madhavi D. A modified liquid chromatographic method development and validation for simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in bulk and tablet dosage form. Int J Pharm Sci Res 2016;7:2996-3001.
18. Vora DN, Kadav AA. Development and validation of a simultaneous HPLC method for estimation of bisoprolol fumarate and amlodipine besylate from tablets. Indian J Pharm Sci 2008;70:542-6.
19. Braza AJ, Modamio P, Lastra CF, Mariño EL. Development, validation and analytical error function of two chromatographic methods with fluorimetric detection for the determination of bisoprolol and metoprolol in human plasma. Biomed Chromatogr 2002;16:517-22.
20. Duan HB, Cao JT, Wang H, Liu YM. Determination of metoprolol tartrate and bisoprolol fumarate by capillary electrophoresis coupled with tris (2,

2'-bipyridyl)-ruthenium (ii) electrochemiluminescence detection and study on the interaction between the drugs and human serum albumin. Anal Methods 2015;7:3946-51.
21. Wang J, Zhang X, Pi F, Wang X, Yang N. Tris (2, 2′-bipyridyl) ruthenium (II)–bisoprolol-based electrochemiluminescence coupled with capillary zone electrophoresis. Electrochim Acta 2009;54:2379-84.
22. Gagyi L, Gyéresi Á, Kilár F. Role of chemical structure in stereoselective recognition of β-blockers by cyclodextrins in capillary zone electrophoresis. J Biochem Biophys Methods 2008;70:1268-75.
23. Ghante MR, Akhade N, Gota P, Nikam A, Jagtap S, Nikam V. Development and validation of high-performance thin-layer chromatography method for simultaneous estimation of nebivolol hydrochloride and cilnidipine. Asian J Pharm Clin Res 2019;12:347-50.
24. Rathod RH, Patil AS, Shirkhedkar AA. Novel NP and RP-HPTLC in praxis for simultaneous estimation of chlorthalidone and cilnidipine in bulk and pharmaceutical formulation. Anal Chem Lett 2018;8:862-71.
25. Shah DM, Doshi DB. Development and validation of HPTLC method for simultaneous estimation of nebivolol hydrochloride and cilnidipine in combined pharmaceutical tablet dosage form. Int J Pharm Res Rev 2016;5:1-7.
26. Dedhiya PP, Patel CJ, Chauhan RS, Kalyankar G, Vyas R, Shah SA. Development and validation of HPTLC method for simultaneous estimation of olmesartan medoxomil and cilnidipine in their combined pharmaceutical dosage forms. J Pharm Appl Sci 2015;2:21-7.
27. Minase AS, Dole MN. Development and validation of analytical method for simultaneous estimation of cilnidipine and olmesartan medoxomil in bulk and tablet dosage form by HPTLC. J Adv Sci Res 2014;5:34-8.
28. Karmalkar HS, Vaidya VV, Gomes NA, Choukekar MP, Kekare MB. Determination of cilnidipine from pharmaceutical formulation by high performance thin layer chromatographic method. Anal Chem 2008;7:573-6.
29. Swapna G, Rahaman SA, Rani AP. Development and validation of stability indicating analytical method for simultaneous estimation of cilnidipine, chlorthalidone and telmisartan in bulk and tablet dosage form. Indian Drugs 2020;57:51-5.
30. Pandya HB, Patel PB. Development and validation of analytical methods for the simultaneous estimation of metoprolol succinate, chlorthalidone and cilnidipine in tablet dosage form. World J Pharm Pharm Sci 2018;7:945-63.
31. Prajapati KM, Patel B, Patel J. Stability indicating RP-HPLC method development and validation for simultaneous estimation of cilnidipine and irbesartan in its pharmaceutical dosage form. World J Pharm Pharm Sci 2017;6:1017-26.
32. Kachave N, Kale M, Wagh D. Simultaneous estimation of cilnidipine and valsartan by RP-HPLC in tablet formulation. Eur J Anal Chem 2016;11:245-53.
33. Hinge MA, Desai DK, Patel ES. Simultaneous estimation of cilnidipine and metoprolol succinate by RP-HPLC. Scholars Res Libr 2015;7:333-40.
34. Patel PR, Patel N, Shah SK. Analytical method development and validation for simultaneous estimation of nebivolol hydrochloride and cilnidipine in combined dosage form. J Chem Pharm Res 2015;7:951-60.
35. Kalyankar GG, Patel J, Bodiwala KB, Lodha SR, Mistry V. Development and validation of first order UV derivative spectroscopy method for simultaneous estimation of cilnidipine and chlorthalidone in their combined tablet dosage form. Pharm Sci Monit 2019;10:101-11.
36. Reddy BH, Spandana B, Mounika D, Devi S, Dacha A, Prakash KV. Simultaneous estimation of telmisartan, chlorthalidone and cilnidipine by absorbance correction method using UV spectrophotometry. Indo Am J Pharm Res 2018;5:1998-2003.
37. Patel SN, Hinge MA, Bhanushali VM. Development and validation of an UV spectrophotometric method for simultaneous determination of cilnidipine and chlorthalidone. J Pharm Res 2015;9:41-5.
38. Agrawal O, Bansod T. Simultaneous estimation of cilnidipine and metoprolol succinate by UV-spectrophotometric and RP-HPLC method in bulk and solid dosage. Invent Impact Pharm Anal Qual Assur 2016;4:196-204.
39. Hinge MA, Desai DK, Patel ES, Singh R, Chavda R, Patel D. Development and validation of UV spectrophotometric method for simultaneous estimation of cilnidipine and metoprolol succinate in bulk drugs and combined dosage form. Der Pharm Lett 2015;7:299-306.
40. Buchiya FV, Bhim AI, Raj HA, Jain VC. Simultaneous determination of cilnidipine and valsartan in synthetic mixture using spectrophotometric technique. Asian J Pharm Anal 2015;5:21-5.
41. Hetal VP, Tulsi A, Pinkal P. Development and validation of first order derivative UV spectrophotometric method for simultaneous estimation of valsartan and cilnidipine in combination. J Pharm Res 2015;4:108-11.
42. Haripriya M, Antony N, Jayasekhar P. Development and validation of UV spectrophotometric method for the simultaneous estimation of cilnidipine and telmisartan in tablet dosage form utilising simultaneous equation and absorbance ratio method. Int J Pharm Biol Sci 2013;3:343-8.
43. Thula KC, Patel DC, Maheshwari DG. Development and validation of first order derivative UV spectrophotometric method for simultaneous estimation of nebivolol and cilnidipine in pharmaceutical formulation. Int J Pharm Sci Rev Res 2015;31:243-7.
44. El Hamd MA, Derayea SM, Abdelmageed OH, Askal HF. Spectrophotometric method for determination of five 1, 4-dihydropyridine drugs using N-bromosuccinimide and indigo carmine dye Int J Spectrosc 2013;2013:243059.
45. Chaudhari SR, Shirkhedkar AA. An investigative review

for pharmaceutical analysis of 1, 4-dihydropyridine-3, 5-dicarboxylic acid derivative: Cilnidipine. Crit Rev Anal Chem 2021;51:268-77.
46. Patel H, Damahe DP, Narkhede SB. RP-HPLC method development and validation for simultaneous estimation of cilnidipine and bisoprolol fumarate in tablet dosage form. Int J Chem Tech Res 2019;12:269-76.
47. Kadia TK, Shah DB, Dilip GM. Development and validation of Q-absorbance ratio spectrophotometric method for simultaneous estimation of cilnidipine and metoprolol succinate in bulk and combined dosage form. Int J Pharm Pharm Sci 2014;6:401-7.

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