Original Article

SIMULTANEOUS DETERMINATION OF CARVEDILOL AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORM BY FIRST ORDER DERIVATIVE UV SPECTROPHOTOMETRY

AUDUMBAR DIGAMBAR MALI*

Department of Pharmaceutics, Sahyadri College of Pharmacy, Methwade, Sangola 413307, Solapur, Maharashtra, India
Email: maliaudu442@gmail.com

ABSTRACT

Objective: To develop an accurate, precise and linear UV spectrophotometry method for simultaneous determination of Carvedilol and Hydrochlorothiazide in Co-Dilatrol® tablet and validate as per ICH guidelines.

Methods: Derivative spectrophotometric methods: The amplitudes in simultaneous determination of the first order derivative of the resultant spectra at 301 nm and at 278 nm were selected to find out Carvedilol and Hydrochlorothiazide respectively in its tablet dosage form by using methanol as a solvent.

Results: The linearity was found to be 5-25 μg/ml for Carvedilol and Hydrochlorothiazide. The mean % recoveries were found to be 101.13% and 99.02% for simultaneous determination of first order derivatives method of Carvedilol and Hydrochlorothiazide. For Intraday precision, Inter day precision % RSD was found to be 0.0082, 0.6304 and 0.0096, 0.6354 for Carvedilol and 0.0085, 0.6257 and 0.0083, 0.6398 for Hydrochlorothiazide respectively. Limit of Detection and Quantitation was found to be 0.7852μg/ml and 2.2539μg/ml for Carvedilol and 0.7859μg/ml and 2.3571μg/ml for Hydrochlorothiazide. Assay results of market formulation were found to be 100.89% for simultaneous determination of the first order derivatives method of Carvedilol and Hydrochlorothiazide. The proposed method has been validated as per ICH guidelines and successfully applied to the simultaneous determination of the first order derivatives method of Carvedilol and Hydrochlorothiazide in its tablet dosage form.

Conclusion: A simple, accurate, precise, linear and rapid UV spectrophotometry method was developed for simultaneous determination of Carvedilol and Hydrochlorothiazide in Co-Dilatrol® tablet and validated as per ICH guidelines. Hence it can be used for the routine analysis of Carvedilol and Hydrochlorothiazide in tablets in various pharmaceutical industries.

Keywords: Carvedilol, Hydrochlorothiazide, UV visible spectrophotometry, Method Validation, First order derivative method.

INTRODUCTION

Carvedilol is a combined alpha-and nonselective betablocker. Carvedilol chemically, 2-Propanol, 1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxy phenoxy) ethyl amino]-, (±)-; (±)-1-(Carbazol-4-yloxy)-3-[[2-(o-methoxy phenoxy) ethyl] amino]-2-propanol. It is a non-selective beta blocker indicated in the treatment of mild to moderate congestive heart failure (CHF). [1, 2] It blocks beta-1 and beta-2 adrenergic receptors as well as the alpha-1 adrenergic receptors. Carvedilol is official drug in British Pharmacopoeia. It has been prescribed as an antihypertensive agent and an angina agent. It is first beta blocker labeled in United States especially for the treatment of heart failure of ischemic or cardio myopathic origin with significant antioxidant activity.

Relative to other beta blocker, carvedilol (CAR) has minimal inverse agonist indicating a reduced negative chronotropic and inotropic effect, which decrease its potential to worsen symptoms of heart failure. At high dosage, it exerts calcium channel blocking activity. The benefits of using CAR in patient with CHF in both single center and multi center trial have been reported in the literature. It prevents vitamin E, glutathione and SH protein depletion induced by oxidation stress, the main defense mechanism against tissue injury caused by free radical. Literature survey revealed the estimation methods of Carvedilol or with other drugs by UV spectrophotometry [3], HPLC [4, 5], calirimetric methods, flow injection analysis, and HPTLC.

Hydrochlorothiazide chemically known as 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide-1,1-dioxide is a moderately potent thiazide diuretic [9, 10]. It exerts its effect by reducing the reabsorption of electrolytes from the renal tubules, thereby increasing the excretion of sodium and chloride ions, and consequently of water. Hydrochlorothiazide is used in the treatment of hypertension either alone or with other antihypertensives [6]. Literature survey revealed the estimation methods of

Hydrochlorothiazide or with other drugs by UV spectrophotometry [7-9] HPLC [10, 11] calirimetric method, flow injection analysis and HPTLC.

Application of derivative technique of spectrophotometry offers a powerful tool for quantitative analysis of multi-component mixtures. When derivatives, the maxima and minima of the original function take zero values, and the inflections are converted into maxima or minima, respectively. The derivative curves are more structured than the original spectra, thus enabling very tiny differences between the original spectra to be identified. Derivative spectrophotometry provides selectivity and offers a solution in resolving the overlapping spectra in multi-component analysis without previous chemical separation.

In the last decades, this technique has rapidly gained an application in the field of pharmaceutical analysis to overcome the problem of interference, due to substances other than analytes, commonly present in pharmaceutical formulations or for the combination of two or more drug substances.

Lack of any published method for simultaneous spectrophotometric determination of Carvedilol and Hydrochlorothiazide, therefore, provoked us to investigate the application of derivative spectrophotometry for simultaneous determination of these compounds in pharmaceutical dosage forms using zero-crossing method [12, 13].

MATERIALS AND METHODS

Apparatus and instrumentation

A shimadzu 1800 UV/VIS double beam spectrophotometer with 1 cm matched quartz cells were used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an

Ultrasonic Cleaning Bath (Spectra lab UCB 40, India). Calibrated volumetric glassware (Borosil®) was used for the validation study.

Materials

Reference standard of Carvedilol and Hydrochlorothiazide API was supplied as gift sample by Lupin Laboratory park Aurangabad, Maharashtra, India. The commercial formulation Co-Dilatrol® as purchased from the local market Solapur, Maharashtra, India.

Method development

Preparation of standard stock solution

Stock solution was prepared by diluting 10 mg of each drug in sufficient quantity of methanol in separate volumetric flask and volume was made up to 100 ml to get the concentrations of 100 μg/ml for each drug. Dilutions from stock solution were prepared in the range of 5-25 μg/ml for Carvedilol and 5-25 μg/ml for Hydrochlorothiazide. Methanol was used as a blank solution.
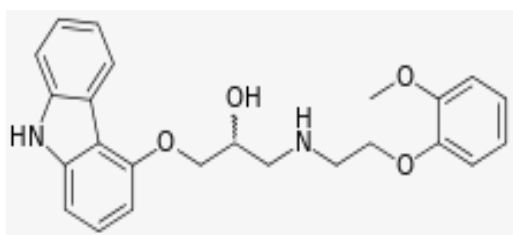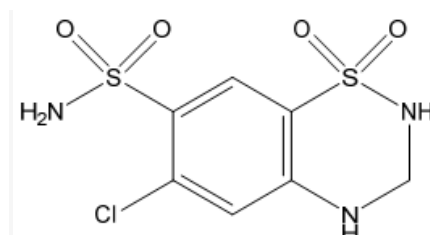

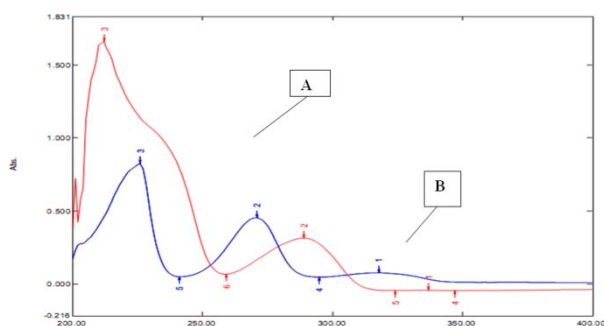**Fig. 1: Chemical structure of Carvedilol**

Fig. 3: Zero order spectra (overlain) of Carvedilol 20 μg/ml (A) and Hydrochlorothiazide 20 μg/ml (B)
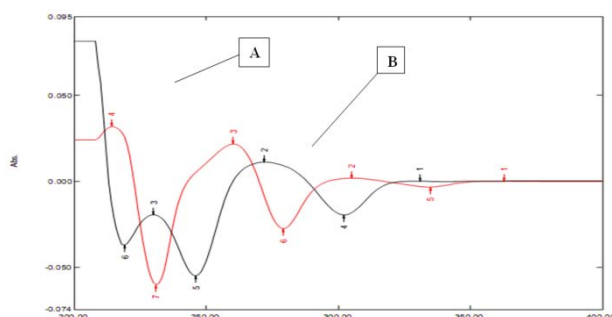

Fig. 4: First order derivative spectra (overlain) of Carvedilol 20 μg/ml (A) and Hydrochlorothiazide 20 μg/ml (B)

Analysis of commercial tablet formulation

Contents of 20 tablets were weighed and their average weight was determined and powdered. Accurately weighed powder equivalent to fill weight of one tablet was transferred to 100 ml calibrated flask containing 50 ml of methanol and sonicated for 30 minutes. The volume was then made up to the mark with methanol. The resulting

Spectrophotometric measurements

Zero-order spectra of standard solutions of Carvedilol (20μg/ml) and Hydrochlorothiazide (20 μg/ml) versus their solvent blank were recorded in the range of 200-400 nm (fig. 3). The first order derivative spectra of these solutions were obtained in the same range of wavelength against their blanks (fig. 4).

The values of first order derivative amplitudes for Carvedilol in the presence of Hydrochlorothiazide and vice versa were measured at 301 nm (zero-crossing of Carvedilol) and 278 nm (zero-crossing of Hydrochlorothiazide), respectively. The calibration curves for derivative spectrophotometry were constructed by plotting the drug concentration versu*s* the absorbance values of the first order derivative spectrum, at 301 nm for Carvedilol and at 278 nm for Hydrochlorothiazide [14, 15].

Fig. 2: Chemical structure of Hydrochlorothiazide

solution was then filtered through what mann filter paper (#41). From this solution, 1 ml was transferred to another 10 ml calibrated flask and diluted up to 10 ml which gives 200 μg/ml concentration of the solution. Then 1 ml of this solution was further diluted to 10 ml to get approximate concentration 20 μg/ml of Carvedilol and 20 μg/ml of Hydrochlorothiazide.

Table 1: Assay of tablet dosage form

S. No.	Sample solution concentration (μg/ml)	Amount found (%)*	Mean % found	%RSD
1	20	99.47		
2	20	102.92	100.89	0.5421
3	20	100.28		

Note:-*n=3, % RSD = % Relative standard deviation.

RESULTS AND DISCUSSION

The proposed simultaneous determination method provides simple, specific, precise, accurate and reproducible quantitative analysis for determination of Carvedilol and Hydrochlorothiazide in Co-Dilatrol® tablets. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The proposed method can be used for routine analysis and quality control assay of Carvedilol and Hydrochlorothiazide in bulk and tablet formulations.

Linearity and range

Linearity

Calibration curves were constructed using six replicates of Carvedilol solutions between 5-25 μg/ml in the presence of 5-25 μg/ml of Hydrochlorothiazide. The same procedure was used for solutions containing Hydrochlorothiazide 5-25 μg/ml in the presence of 5-25 μg/ml of Carvedilol. The calibration curves were constructed (fig. 5 and fig. 6) and statistical analysis were

performed. The regression equations of calibration curves were y=0.017x+0.001 (r²=0.9997) at 301 nm for Carvedilol and y=0.016x+0.005 (r²=0.9994) at 278 nm for Hydrochlorothiazide for first order derivative spectrophotometry methods. The range was found to be 5-25 µg/ml for both drugs for second order spectrophotometry methods.
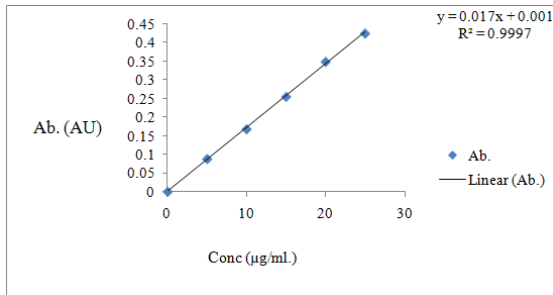

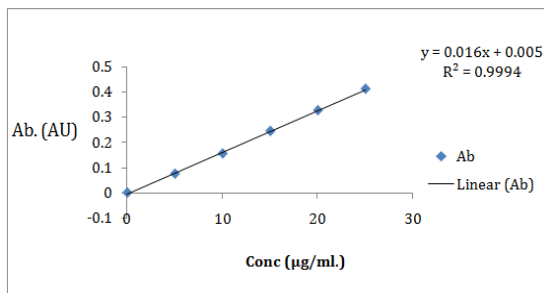**Fig. 5: Calibration curve for carvedilol at 301 nm**

Fig. 6: Calibration curve for hydrochlorothiazide at 278 nm

Table 2: Stastical data for the calibration graphs for determination of carvedilol and hydrochlorothiazide by proposed methods

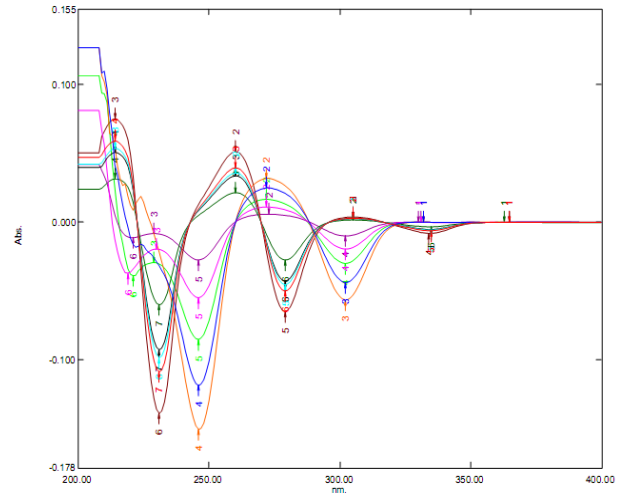| Parameters	Carvedilol	Hydrochlorothiazide
Linearity range (µg/ml)*	5-25	5-25
r²±SD*	0.9997	0.9995

Note:-*n=3, SD.: Standard deviation

Fig. 7: First order derivative overlay of carvedilol and hydrochlorothiazide at 5, 10, 15, 20 and 25 µg/ml concentrations

Accuracy

For accuracy determination, the analysed samples were spiked with extra 80%, 100% and 120% of the standard solution of both drugs and the mixtures were re analysed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the commercial tablet formulations. The mean recoveries and % RSD are illustrated in table 3. The data indicates that the proposed derivative spectrophotometric method is highly reproducible during one run and between different runs.

Precision

To determine the precision of the method, Carvedilol and Hydrochlorothiazide solutions at a concentration of 20µg/ml were analysed each three times for first order spectrophotometric method. Solutions for the standard curves were prepared fresh every day.

Table 3: Results of drug content and analytical recovery of carvedilol and hydrochlorothiazide

Parameters	Carvedilol	% RSD	Hydrochlorothiazide	% RSD
Labelled claim	25 mg	-	12.5 mg	-
% Drug content±SD	101.13±0.1302	0.36	99.02±0.1982	0.39
Analytical recovery at 80 %±SD	99.58±0.2854	0.14	100.14±0.4596	0.17
Analytical recovery at 100 %±SD	100.02±0.1335	0.24	100.01±0.4258	0.29
Analytical recovery at 120%±SD	101.11±0.5279	0.19	99.07±0.5811	0.16

Table 4: Results of intra and inter day precision

Parameters	Intraday precision		Inter day precision	
	SD*	% RSD*	S. D*	% RSD*
Carvedilol	0.0082	0.6304	0.0096	0.6354
Hydrochlorothiazide	0.0085	0.6257	0.0083	0.6398

Note:-*n=3, SD: standard deviation

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3xσ/S and LOQ = 10xσ/S, where σ is the standard deviation of intercept, S is the slope.

The LOD and LOQ were found to be 0.7852µg/ml and 2.3539µg/ml respectively of Carvedilol for first order derivative and 0.7859µg/ml and 2.3571µg/ml respectively of Hydrochlorothiazide for the first order derivative.

Analysis of the marketed formulation

There was no interference from the excipients commonly present in the tablets. The drug content was found to be 100.89% first order spectrophotometric methods. It may therefore be inferred that degradation of Carvedilol and Hydrochlorothiazide had not occurred in the marketed formulations that were analysed by this method. The low % RSD value indicated the suitability of this method for routine analysis of Carvedilol and Hydrochlorothiazide in pharmaceutical dosage form.

Table 5: Summary of validation parameters

Parameter	Carvedilol	Hydrochlorothiazide
λ range	200-400 nm	200-400 nm
Regression Equation (y=mx+c)	Y=0.017x+0.001	Y=0.016x+0.005
Measured wavelength	301 nm	278 nm
Linearity range	5-25µg/ml	5-25µg/ml
Slope	0.017	0.016
Intercept	0.001	0.005
Correlation coefficient (R^2)	0.9997	0.9994
Limit of Detection (LOD) µg/ml	0.7852	0.7859
Limit of Quantitation (LOQ) µg/ml	2.2539	2.3571
Accuracy (Mean % Recovery)	101.13	99.02
Precision (%RSD)	0.36	0.39

CONCLUSION

From the results of this study it can be concluded that the proposed first order derivative spectrophotometric method can be used for simultaneous determination of Carvedilol and Hydrochlorothiazide. This method is simple, rapid, practical, reliable and inexpensive and can be used for routine analysis of simultaneous determination of these compounds without any prior separation in quality control laboratories.

ACKNOWLEDGEMENT

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Mali Audumbar, Nagargoje Rajendra, Hake Gorakhnath, Tamboli Ashpak. Zero order and area under curve spectrophotometric methods for determination of carvedilol in pharmaceutical formulation. Inventi Rapid Pharm Anal Quality Assurance 2015;2:1-5.
2. Rajan V Rele, Prathamesh P Tiwatane. UV Spectrophotometric estimation of carvedilol hydrochloride by first order derivative and area under curve methods in bulk and pharmaceutical dosage form. Pharm Sin 2014;5:29-35.
3. Ansary A, Abdel-Moety MM, Abdel Gawad FM, Ether AM, Khater MM. Simultaneous determination of carvedilol and hydrochlorothiazide in tablets and spiked human plasma using derivative spectrophotometry. Pharm Anal Acta 2012;3:1-6.
4. Belal TS, Shaalan RA, El Yazbi FA, Elonsy SM. Validated stability-indicating HPLC-DAD determination of antihypertensive binary mixture of carvedilol and hydrochlorthiazide in tablet dosage forms. Chromatographia 2013;76:1707-20.
5. Alzoman NZ, Sultan MA, Maher HM, Al-Shehri MM, Ola IV. Validated stability-indicating capillary electrophoresis method for the separation and determination of a fixed-dose combination of carvedilol and hydrochlorothiazide in tablets. J AOAC Int 2013;96:951-9.
6. Patel HM, Pancholi SS, Jivani NP. UV Spectrophotometric method for simultaneous estimation of carvedilol and hydrochlorthiazide in bulk and pharmaceutical dosage form by simultaneous equation method. Inventi Rapid Pharm Anal Quality Assurance 2013;1:49-56.
7. LJ Patel, BN Suhagia, PB Shah, RR Shah. Rp-Hplc and hptlc methods for the estimation of carvedilol in bulk drug and pharmaceutical formulations. Indian J Pharm Sci 2006;68:790-3.
8. Hapse SA, Wagh VS, Kadaskar PT, Dokhe MD, Shirsath AS. Spectrophotometric estimation and validation of hydrochlorothiazide in tablet dosage forms by using different solvents. Scholars Res Library Pharm Chem 2012;4:10-4.
9. Venkatesan Subramanian, Kannappan Nagappan. Analytical method development and validation of telmisartan and hydrochlorothiazide in tablets using orthogonal polynomial function method. Int J Pharm Pharm Sci 2013;5:73-5.
10. Vrushali Tambe, Vijaya Vichare, Ujjawala Kandekar, Shashikant Dhole. Novel Uv spectrophotometric methods for estimation of ramipril and hydrochlorothiazide by simultaneous equation and area under curve method. Int J Appl Pharm 2010;2:20-2.
11. Sachin Bhagwate, NJ Gaikwad. Stability Indicating HPLC method for the determination of hydrochlorothiazide in pharmaceutical dosage form. J Appl Pharm Sci 2013;3:88-92.
12. T Gopala Swamya, K Nagarajub, A Lakshmana Rao. Rp-Hplc Method for the simultaneous estimation of telmisartan and hydrochlorothiazide in pharmaceutical dosage form. Int J Drug Dev Res 2011;3:362-8.
13. Audumbar Digambar Mali. Zero, First, Second order derivative and area under curve spectrophotometric methods for determination of cefixime trihydrate in pharmaceutical formulation. Int J Pharm Pharm Sci 2015;7:321-5.
14. Subramaniyanvenkatesan, Nagappankannappan. Development and validation of spectrophotometric method using vierordt's method for simultaneous estimation of moxifloxacin and cefixime in tablet formulation. Int J Pharm Pharm Sci 2013;5:768-72.
15. International Conference on Harmonization (ICH) of Technical Requirements for the registration of Pharmaceuticals for Human use, Validation of Analytical Procedures Methodology. ICH-Q2 (R1), Geneva; 1996. p. 1-8.