INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

DEVELOPMENT AND VALIDATION OF RP-UPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CARVEDILOL AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORM

G.Sivakamasundari [1]*, N.Kannappan [2]
[1]Assistant professor, Department of Pharmacy, Annamalai University, Annmalai Nagar, Tamil Nadu, India
[2]Associate professor, Department of Pharmacy, Annamalai University, Annmalai Nagar, Tamil Nadu, India
*Corresponding Author Email: sivakami.space@gmail.com

Article Received on: 27/12/17 Approved for publication: 02/02/18

DOI: 10.7897/2230-8407.09221

ABSTRACT

The aim of the present study was to develop and validate a simple, novel, rapid isocratic ultra-performance liquid chromatography for simultaneous estimation of Carvedilol and Hydrochlorothiazide in combined dosage form using Hypersil gold Column - C18 (50 X 2.1mm) 1.9 micron with Mobile phase composition of Water, methanol, and Acetonitrile in the ratio of 30:20:50 (pH 5.5). The flow rate of 0.3 ml/ min and UV detection at 285 nm was maintained during entire study. The retention time of hydrochlorothiazide and Carvedilol were found to be 2.06 and 2.39 mins respectively. All validation parameters were evaluated as per ICH guideline, which remained well within the acceptable limit. This proposed method can be used for estimation of Carvedilol and Hydrochlorothiazide in bulk and pharmaceutical dosage forms

Keywords: Carvedilol, Hydrochlorothiazide, Tablet, UPLC, method Validation

INTRODUCTION

The present study was performed to separate and quantify Carvedilol and Hydrochlorothiazide in combined pharmaceutical dosage form by using RP-UPLC technique.

Carvedilol (Figure.1), is a non-cardio selective beta blocker. Chemically it is (2RS)-1-(9H-carbazol-4-yloxy)-3-[[2-(2-ethoxyphenoxy) ethyl] amino] propan-2-ol. It is official in European Pharmacopoeia and British Pharmacopoeia[1]. Carvedilol is used in the management of hypertension, angina pectoris and as an adjunct to standard therapy in symptomatic heart failure. Hydrochlorothiazide (Figure.2), is a moderately potent diuretic. Chemically it is 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide-1, 1-dioxide.it is official in United States Pharmacopeia, European Pharmacopoeia, Indian Pharmacopeia[2,3]. It is used in the treatment of hypertension, edema associated with heart failure and with renal and hepatic disorders. The Carvedilol and hydrochlorothiazide were estimated by UV spectrophotometry methods[4, 5] HPLC methods[6, 7]. Few methods are available for simultaneous determination of Carvedilol and hydrochlorothiazide[8-10].

To the best of our knowledge, there are no reports available on RP-UPLC method for Carvedilol and hydrochlorothiazide in combined dosage form with short run time. It is therefore, felt necessary to develop a new method for simultaneous determination of both the drugs with shorter run time. We intend to opt for a faster chromatographic technique UPLC, for the said study. The objective of the work was directed toward the development, validation and application of simple and easy to use method for routine determination of the studied drugs in their formulation. As method validation is an important requirement in analytical method development, the proposed method has been validated as per ICH guidelines.

MATERIALS AND METHODS

Chemicals

The reference sample of Carvedilol and Hydrochlorothiazide were procured from Ideal Laboratory, Pondicherry, India and Codilatrol brand Tablet having combination of Carvedilol and Hydrochlorothiazide 25 mg and 12.5 mg was obtained from market. HPLC grade Methanol and Acetonitrile (Mfg by: S.D. Fine Chemicals, Mumbai), Acetic acid AR Grade (Qualigens fine chemicals, Mumbai) Triethylamine of HPLC grade (Thermo Fischer scientific Ltd) were used. Water was collected from Milli Q purification unit with 0.22 µ filters.

Instrument

The analysis was carried out on a THERMO-SCIENTIFIC UPLC system (thermo-scientific, USA) equipped with Column C18 (50 X 2.1mm) 1.9micron (Thermo scientific - Hypersil gold) and ACCELA-1250 pump with auto sampler and a photo diode array detector. Data acquisition, data handling and instrumentation control were performed by CHROM-QUEST software was used to optimize the Method.

Methods

Method Optimization

At the initial stage different columns like c_8 and c_{18} with particle size of 1.7µ were tried with mobile phase using different buffer with different molar concentration in the pH range 3 to 8. Finally, Column- C18 (50 X 2.1mm) 1.9micron and mixture of Water, Methanol, Acetonitrile in the ratio of (30:20:50) and, pH 5.5 was adjusted with acetic acid and 1ml Triethylamine was selected. The solvent mixture was filtered through a 0.22 µ PVDF filter and

sonicated before use. The flow rate was 0.3 ml/min. The wavelength detection of the drug was monitored at 285 nm. The optimized chromatogram is shown in Figure 3[11].

Mobile Phase Preparation

Mobile phase was prepared by dissolving 200 ml of Methanol and 500 ml Acetonitrile in 300 ml of Milli Q water.3 ml of Acetic acid was to adjust the PH 5.5 and 1ml Triethylamine was added. The mobile phase was filtered through 0.2µ filter and degassed in an Ultrasonication

Preparation of Standard Solution

25 mg of hydrochlorothiazide working standard was weighed accurately and transferred into 100 ml volumetric flask. About 50 ml of methanol was added and sonicated for dissolving and volume was made up to the mark with diluent and mixed well (solution A)
50 mg of Carvedilol working standard was weighed and transferred into 100 volumetric flasks. 50 ml methanol was added and sonicated for complete dissolving and volume was made up to the mark with diluent and mixed well solution B)
Pipetted 1 ml of Hydrochlorothiazide stock solution (solution A) and 1 ml of Carvedilol stock solution (solution B) into 25 ml volumetric flask. Volume was made up to the mark with Mobile phase and mixed well

Sample Preparation

Ten tablets containing Carvedilol and Hydrochlorothiazide as active ingredient were weighed and finely powdered. 455 mg of Tablet powder weighed, transferred into 100 ml volumetric flask, diluted with Methanol, sonicated for 30 minutes and volume made up to the mark. From this solution 1ml was transferred into 25ml volumetric flask. Volume was made up to 25 ml with Mobile phase and mixed well.

Method validation

In the optimized condition, the validation parameters were validated as per International Conferences on Harmonization (ICH) guidelines

Specificity

Specificity was performed by injecting blank and standard preparations. Chromatograms were recorded and retention times of sample and standard preparations were compared for identification of analytes. Individual Chromatograms are shown in Figure 4, 5.

Table 1: System Suitability Parameters for Carvedilol and Hydrochlorothiazide

Parameters	Carvedilol	Hydrochlorothiazide
Retention time	2.397	2.068
Theoretical plates	5388	5245
Tailing factor	1.23	1.21

Table 2: Recovery Studies of Carvedilol and Hydrochlorothiazide

Level of recovery (%)	100		110		120		130	
	car	hyd	car	Hyd	car	hyd	Car	hyd
Amount present (ug)	20	10	20	10	20	10	20	10
Amount of std.added (ug)	0	0	2	1	4	2	6	3
%Recovery	100.09	99.99	100.40	101.20	100.81	100.11	100.25	100.45

Table 3: Statistical Validation of Recovery Study

Levels of recovery (%)	Drug	Mean% recovery	Standard deviation	%RSD
100	Carvedilol	100.22	0.329	0.32
	hydrochlorothiazide	100.23	0.55	0.54
110	Carvedilol	100.19	0.578	0.57
	hydrochlorothiazide	100.24	0.292	0.28
120	Carvedilol	99.88	0.248	0.24
	hydrochlorothiazide	100.28	0.317	0.31
130	Carvedilol	99.7	0.258	0.25
	hydrochlorothiazide	100.07	0.113	0.11

Table 4: Precision data of Carvedilol and Hydrochlorothiazide

Compound (n=6)	Intraday precision		Interday precision	
	%Amount found	%RSD	%Amount found	%RSD
Carvedilol	99.68	0.106	99.67	0.252
Hydrochlorothiazide	99.87	0.2	100.03	0.317

Table 5: Robustness study of Carvedilol and Hydrochlorothiazide

Parameters	Amount found %(mean±SD)		%RSD	
	Car	Hyd	Car	Hyd
Flow rate (0.285ml)	99.5±0.12	100.3±.32	0.11	0.31
Flow rate (0.3ml)	100.2±0.23	99.2±.23	0.24	0.31
Flow rate (0.315ml)	100.3±0.32	100.2±0.54	0.35	0.58
Wavelength 283	99.89±0.44	100.2±0.84	0.45	0.87
Wavelength 284	100.05±0.11	100.02±0.66	0.11	0.68
Wavelength 285	100.5±0.35	99.81±0.24	0.37	0.25
Wavelength 286	99.5±±0.54	100.2±0.60	0.55	0.61
Wavelength 287	99.9±0.26	100.02±0.21	0.26	0.25

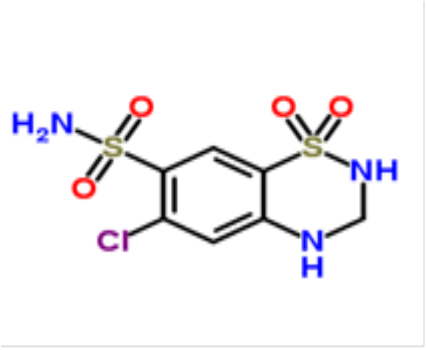

Figure 1: Structure of Hydrochlorothiazide

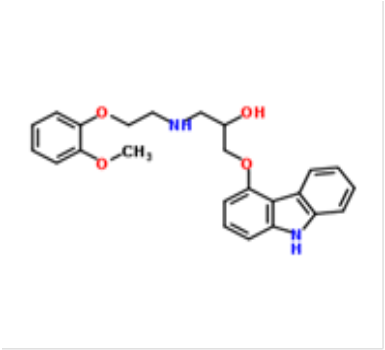

Figure 2: Structure of Carvedilol

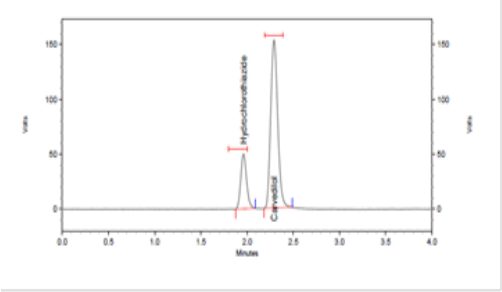

Figure 3: Chromatogram of Hydrochlorothiazide and Carvedilol

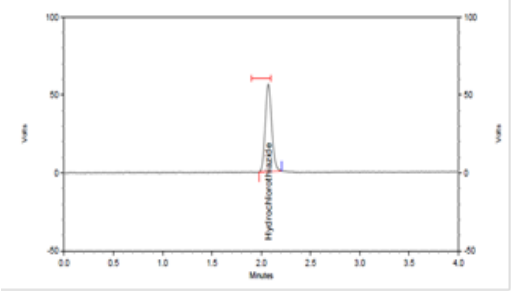

Figure 4: Chromatogram of Hydrochlorothiazide

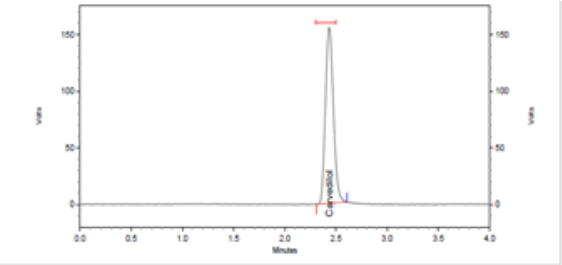

Figure 5: Chromatogram of Carvedilol

RESULTS AND DISCUSSION

The purpose of the present study was to develop and validate the method for estimation of Carvedilol and hydrochlorothiazide. The method was optimized using different combination of mobile phase. In the optimized chromatographic conditions the retention time obtained for the drugs Hydrochlorothiazide and Carvedilol were 2.06 min and 2.39 min respectively.

System Suitability

The system suitability parameters such as retention time, theoretical plate, and tailing factor were carried out and the results are given in the Table 1.

Accuracy

Accuracy of the method was determined by standard addition method by spiking known amount of standard in sample preparation. The percentage recovery at different levels of 100%, 110%, 120% and 130% were calculated. The % recovery was found to be within 99-101%.the results are given in Table 2 and 3.

Precision

The precision of the proposed method was determined by Intraday Precision and Interday Precision. The % RSD was found less than 2. The precision results are given the Table 4.

Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The robustness was checked by making 2 small changes in wavelength detection285±2 nm and flow rate0.3ml±0.15 ml. Overall %RSD was found to be less than 2% for all the variations which indicates that the proposed method is robust. Robustness data is shown in Table 5.

CONCLUSION

In this study a simple RP-UPLC method developed for simultaneous estimation of Carvedilol and Hydrochlorothiazide in pharmaceutical dosage form. Which is specific, precise, accurate, and robust, the shorter run time of within 3 minutes enables rapid determination of the drugs individually or in combination. Satisfactory results were obtained from validation of the method. This method exhibited an excellent performance in terms of sensitivity and speed. The method is more economical and suitable for laboratory use as solvent consumption is very less. Hence the proposed UPLC method could be applied for the routine analysis in quality control approved testing laboratories, Industries, Research institution, Bioequalence studies, and clinical Pharmacokinetics in future.

REFERENCES

1. British Pharmacopeia, The Department of Health and Social Services and Public Safety: Published by British Pharmacopeia Commission, volume I, 2011; 393, 1072.
2. Indian Pharmacopoeia, Indian Pharmacopoeia Commission, Ghaziabad: Government of India, Ministry of Health and Family Welfare, volume 2, 2014; 1284, 1900.
3. United States pharmacopeia and national formulary (usp 31-nf 26), Rockville, United States pharmacopeial convention: Volume 2, 2008; 2334.
4. Dr. C. Theivarasu, Santanu Ghosh and T.Indumathi, UV Spectrophotometric Determination of Carvedilol in Pharmaceutical Formulations. Asian Journal of Pharmaceutical And Clinical Research 2010; Volume 3(4), 64-68 (23-11-2017)
5. Rajan V. Rele and Prathamesh P. Tiwatane, UV spectrophotometric estimation of carvedilol hydrochloride by first order derivative and area under curve methods in bulk and pharmaceutical dosage form. Der Pharmacia Sinica,volume 2014; volume 5(6), 29-35.(23-11-2017)
6. Rohit Bhatia*, Shilpa Katoch and Dharmendra Kumar. Determination of Hydrochlorothiazide and Drugs in its Combination by HPLC, Journal of Chemical and Pharmaceutical Research, 2015;7(12), 184-190 (23-11-2017)
7. Magda M. Ibrahim, Development and validation of a rp-hplc method for the simultaneous determination of Carvedilol, Glimepiride or Glibenclamide in binary combinations; and its application for *in vitro* - interaction studies, Indo American Journal of Pharmaceutical Research, 2015; Volume 5(8), 2791-2794.
8. Audumbar digambar mali,Simultaneous Determination of Carvedilol And Hydrochlorothiazide in Pharmaceutical Dosage form by First Order Derivative UV Spectrophotometry, International Journal of Pharmacy and Pharmaceutical Science 2015;Volume 7(9), 371-374.(25-11-2017)
9. Tarek S. Belal1*, Rasha A. Shaalan1, Application of a new simple Spectrophotometric method for determination of the binary mixtures of hydrochlorothiazide with either carvedilol or losartan potassium in tablets dosage forms, Der Pharma Chemica, 2014; volume 6(4), 120-129 (25-11-2017)
10. Tarek S. Belal, Rasha A. Shaalan, Validated Stability-Indicating HPLC–DAD Determination of the Antihypertensive Binary Mixture of Carvedilol and Hydrochlorothiazide in Tablet Dosage Forms, Chromatographia, 2013; volume 76, 1707–1720 (25-11-2017)
11. M.D.Ashraf Basha, K.Mangamma, G.Manikumar, Method Development and Validation of Telmisartan and Amlodipine Besylate By RP-HPLC in Tablet Dosage Form, Journal of Pharmaceutical and Biomedical Analysis Letters,2014;Volume2(1),55-61

Cite this article as:

G.Sivakamasundari & N.Kannappan. Development and validation of RP-UPLC method for simultaneous estimation of carvedilol and hydrochlorothiazide in pharmaceutical dosage form. Int. Res. J. Pharm. 2018;9(2):23-26 http://dx.doi.org/10.7897/2230-8407.09221

Source of support: Nil, Conflict of interest: None Declared