IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
November 2017 Vol.:10, Issue:4

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Clonidine HCl and Chlorthalidone in Bulk Form

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Hajera N. Khan*, Chopde Asha, Mohammad Zameeruddin, Vishvanath B. Bharkad

Department of Quality Assurance SSS Indira College of Pharmacy, Vishnupuri, Nanded-431606 Maharashtra India.

Submission: 31 October 2017
Accepted: 7 November 2017
Published: 30 November 2017

Keywords: Clonidine HCl, Chlorthalidone, RP-HPLC, Validation.

ABSTRACT

A Reverse phase High-Performance Liquid Chromatography method (HPLC) was developed for the simultaneous estimation of Clonidine HCl and Chlorthalidone in laboratory mixture. The chromatographic separation was achieved by Zorbax Eclipse XDB-C18 (4.6x250mmx5µ) column and Methanol-Ortho Phosphoric Acid (50:50 V/V) was used as mobile phase at a flow rate of 1ml/min. Detection was carried out at 236nm. The retention time of Clonidine HCl and Chlorthalidone were found to be 2.510 min and 3.403 min respectively. The method has been validated for linearity, accuracy, and precision. Linearity observed was 5-25 µg/ml for both drugs. The developed method was found to be accurate, precise and rapid for Simultaneous estimation of Clonidine HCl and Chlorthalidone in laboratory mixture.

INTRODUCTION

Chlorthalidone (CHR) is chemically is 2-chloro-5[(1RS)-1-hydroxy-3-oxo-2, 3-dihydro- 1H-isoindol-1yl) benzene-1-sulfonamide[1]. Chemical structure of chlorthalidone is shown in figure 1. Chlorthalidone is a diuretic drug used to treat hypertension and fluid retention caused by various conditions, including heart diseases. Chlorthalidone is very similar to hydrochlorothiazide and is used as an independent drug or in combination with other antihypertensive agents for lowering arterial blood pressure[2]. Diuretics lower blood pressure by decreasing cardiac output and reducing plasma and extracellular fluid volume[3]

Chemically clonidine HCl (CLD) is chemically ((2-[2, 6-dichlorophenyl] amino)-2-imidazoline) [4] preferentially stimulates central alpha (2)-adrenoceptors. Chemical structure of clonidine is shown in figure 2, which leads to inhibition of sympathetic tone, resulting in a lowering of arterial pressure and of heart rate[5].

Clonidine HCl is a centrally acting alpha-agonist hypotensive agent used to treat hypertension (high blood pressure), attention deficit hyperactivity disorder, migraine etc. Clonidine HCl used to treat psychiatric disorders including stress, sleep disorders, other anxiety disorders. Mild sedative nature of Clonidine HCl implies its use as premedication before surgery or procedures[6].

Literature survey revealed UV-Visible spectrophotometric methods [7, 8] RP-HPLC [9, 10], HPTLC[11] and UPLC[12] Methods for the estimation of CHR and CLD alone or in combination with other drugs. The validation of methods was carried out as per ICH guidelines [13,14]

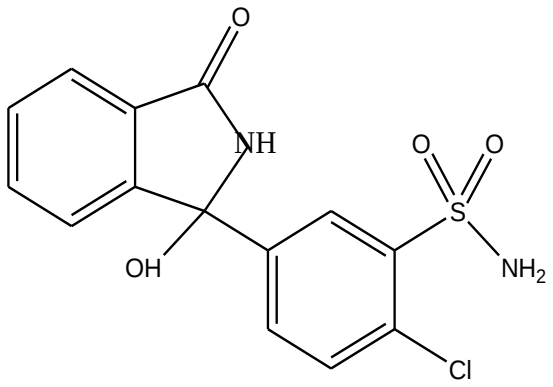

Figure 1. Chlorthalidone

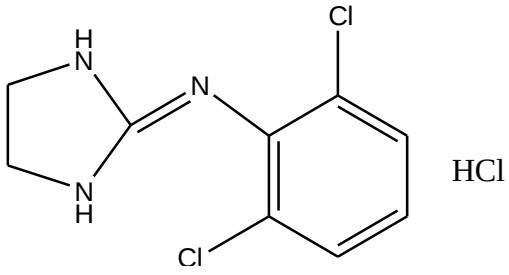

Figure 2. Clonidine Hydrochloride

EXPERIMENTAL

Reagents and Materials

Pure samples of Clonidine HCl and chlorthalidone were provided as gift sample from Neon Lab. Ltd. Mumbai.). HPLC grade Methanol and O-Phosphoric acid procured from Fisher chemicals Ltd India and Merck India respectively.

Chromatographic conditions

The isocratic mobile phase consisted Methanol: O Phosphoric Acid in the ratio of 50:50 (v/v), flowing through the column at a constant flow rate of 1 ml/min. Zorbax Eclipse XDB-C18 column (4.6×250mm x5µ) was used as the stationary phase. By considering the chromatographic parameter, sensitivity, and selectivity of the method for two drugs, 236nm was selected as the detection wavelength for UV detector. The HPLC system was (Agilent 1220 LC).[15]

Selection of Chromatographic Mode

The reverse phase HPLC was selected for separation because it is convenient and rugged than other forms of the liquid chromatography and is more likely to result in a satisfactory final separation.

Selection of Stationary phase

On the basis of reversed phase, HPLC mode and a number of carbon present molecule (analyte) stationary phase with C-18 bonded phase i.e. Zorbax Eclipse XDBC18(4.6□ ~250mm□ ~5µ) with particle size 5 µm was selected.

Selection of Mobile Phase

The selection was made based on literature survey. After assessing the solubility of both drugs in different solvents as well in mobile phases, Methanol: O Phosphoric Acid in ratio 50:50 v/v was selected as a first choice.

Selection of Detection wavelength

By appropriate dilution of a standard stock solution with Methanol Phosphoric Acid (50:50), the 10 µg/ml concentrations of CLD & CHR were prepared. The solution was scanned using double beam UV visible spectrophotometer 1800 in the spectrum mode between the range of 400 nm to 200 nm The analytical wavelength selected was 236nm at which at which drug shows maximum absorbance.

Preparation of standard stock solution

Accurately, about 10 mg of standard CLD & CHR and transferred to separate 100 ml volumetric flasks. The drugs were dissolved in Ethanol then volume made up to the mark with the same solvent to obtain the standard stock solution of each drug of concentration 100 µg/ml.

Preparation of working solution

Take 1ml from the Clonidine HCl stock solution and 1ml from Chlorthalidone stock solution and transferred to 10ml volumetric flask and volume made up to the mark by distilled Water to get 10 µg/ml of each drug.

Method Validation

Linearity

The linearity for Clonidine HCl and Chlorthalidone were assessed by analysis of combined standard solution in the range of 5-25µg/ml respectively for both drugs. Correlation coefficient for calibration curve Clonidine HCl and Chlorthalidone was found to be 0.9986 and 0.9998.

Precision

Intra-day

The intraday precision for Clonidine HCl and Chlorthalidone is shown in table 1. The % RSD for intraday precision was found to be 0.030556 for Clonidine HCl and 0.040425 for Chlorthalidone.

Inter-day

The interday precision for Clonidine HCl and Chlorthalidone is shown in table 2. The % RSD for interday precision was found to be 0.030558 for Clonidine HCl and 0.015276 for Chlorthalidone.

Accuracy

The accuracy of the method was confirmed by recovery study from the marketed formulation at three level of standard addition. The results are shown in table 3. Percentage recovery for Clonidine HCl was 0.110809 and chlorthalidone was 0.195798.

Robustness

Robustness expresses the precision within laboratories, Variation like different solvent. Robustness of the methods was assessed by carrying out assay 3 times with different solvent by using same equipment conditions the % RSD for Robustness was found to be 0.05655 for Clonidine HCl and 0.042422 for Chlorthalidone.

Ruggedness

From stock solution, the sample solution of CLD &CHR(10 µg/mL) was prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions.

Summary of Validation Parameter CLD and CHR

Parameters	CLD	CHR
Linearity range [µg/ml]	5-25µg/ml	5-25µg/ml
Regression equation (Y=mX+C)	Y = 10605x +38395	Y = 637667x +76190
Recovery [% RSD , n = 3]	0.110809	0.195798
Precision [% RSD]		
Intra-day [n = 3]	0.030556	0.040425
Inter-day [n = 3]	0.030556	0.015276
Ruggedness [% RSD] Analyst 1 [n = 3]	0.0028281	0.028281
Robustness	0.05655	0.042422
Specificity	Specific	Specific

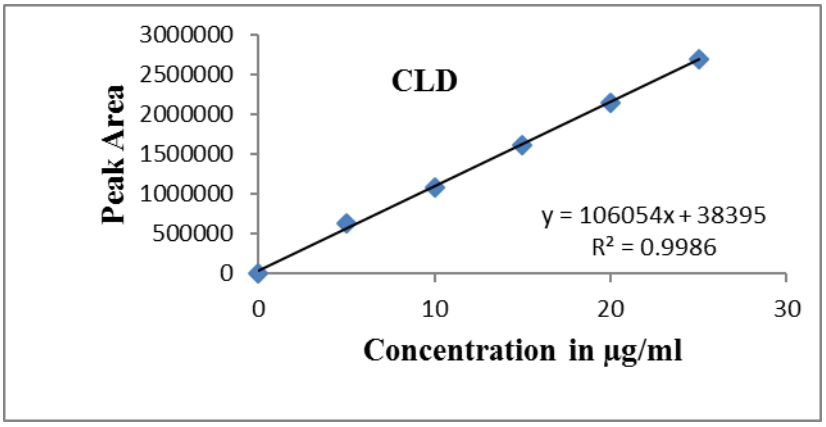

Fig. No. 3: Calibration curve of CLD

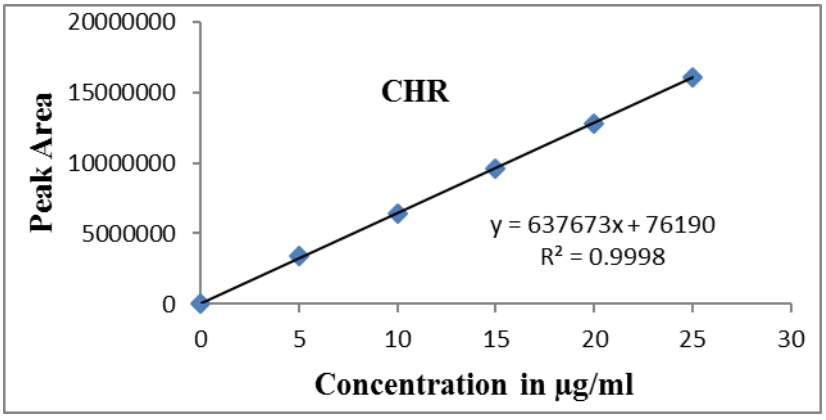

Fig. No. 4: Calibration curve of CHR

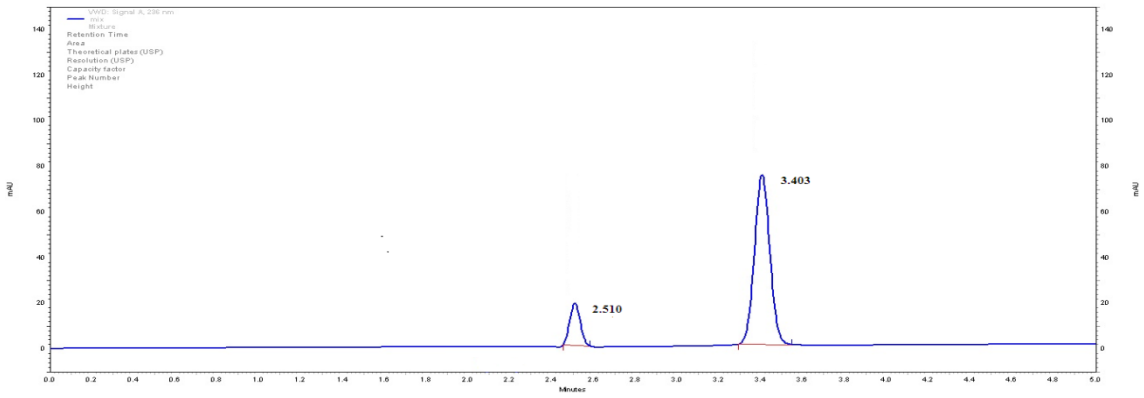

Fig. No. 5: HPLC chromatogram of standard CHR and CLD

RESULTS AND DISCUSSION

HPLC method was developed, validated and used for quantitative determination of Clonidine HCl (CLD) and Chlorthalidone (CHR) from its bulk dosage form. Chromatographic separation was performed on zorbax Eclipse XDB-C18(4.6x250mmx5µ). For the selection of a suitable mobile phase different individual solvents as well as a combination of solvent have been tried by varying aqueous to organic ratio to get a good separation and stable peak. Hence mobile phase Methanol: Ortho Phosphoric Acid (50:50 v/v/), flow rate 1.ml/min, with detection at 236nm and separation was completed in less than 5 min. As per (ICH) guidelines, the method was validated for linearity, accuracy, precision, and robustness. The linearity of Clonidine HCl was found to be in the range of 5-25µg/ml. The correlation was 0.998 & Linearity of Chlorthalidone was found to be in the range of 5-25µg/ml. The correlation was 0.999. The results of bulk drug analysis were found to be 99.91-99.98 with 0.09504-0.025166% standard deviation for the bulk drug. Percent recovery of the bulk drug was found to be 99.40-99.60%. The assay experiment shows that method is free from the interference of excipients, hence the method is specific.

CONCLUSION

Developed HPLC for simultaneous determination of Clonidine HCl and Chlorthalidone was specific and robust. Statistical analysis proves that all the developed methods can be used for routine analysis of said drugs in Laboratory Mixture.

ACKNOWLEDGEMENT

The author is thankful to Neon Lab. Ltd., Mumbai for providing gift sample of Clonidine HCl and Chlorthalidone.

REFERENCES

1. Indian Pharmacopoeia, Government of India Ministry of Health & Family Welfare, 2010, 6th edition Vol II.pp.1076-1077.
2. Tripathi KD. Essential of Medical Pharmacology, 6th edition.Jaypee Brothers Medical Publishers (P) LTD Edn., Japee, 2010,pp.546-47, 565-66.
3. British Pharmacopoeia Commission 2015. British Pharmacopoeia , Vol. I. pp.,530, 597.
4. British Pharmacopoeia Commission. 2008. British Pharmacopoeia Vol I. pp. 570-571, 2555-2556. Stationery Office. London
5. Sweetman, S. C., Blake, P. S. and Parsons, A. V. 2007. Martindale, The Complete Drug Reference, 35th ed. pp.1119-1123. The Pharmaceutical Press, London.
6. United States Pharmacopeial Convention, Inc. 2007. The United States Pharmacopeia XXX. The National Formulary XXV. pp.1798-1800.
7. Padmane SP., Jain ND., Ittadwar AM., Walde SP., 2014.A Derivative UV Spectrophotometric Method For the Simultaneous Determination of Metoprolol Succinate & Chlorthalidone in Combined Dose Tablet Formulation, International Journal of Analytical & Bioanalytical Chemistry, 4(1), 33-41.
8. Parikh P., Sahoo U., Zanvarseth AK., 2013.Derivative Spectrophotometric Method For Simultaneous Estimation of Chlorthalidone & Olmesartan Medoxomil in their Tablet Dosage Form, An International Journal of Pharmaceutical Sciences, 4(4), 111-123.
9. Devanaboyina N., Kumar CB., Bhanu MA., Gayathri V., Vijay B., 2012.Liquid Chromatography Method Development & Validation For Analysis of Clonidine HCl in Pharmaceutical Dosage, Journal of Atoms & Molecules, 2(1), 93-102.
10. Shah KV., Kapupara PP., Gondaliya Khushboom., 2014.Development & Validation of RP-HPLC Method For Simultaneous Estimation of Clonidine HCl & Hydrochlorothiazide in Pharmaceutical Formulation, International Bulletin of Drug Research, 4(6), 106-115.
11. Teli MS, Sawant SS, Shukla S, Simultaneous Analysis of Clonidine HCl & Hydrochlorothiazide in Bulk & In Tablet By HPTLC With Uv Absorption, World Journal of Pharmacy & Pharmaceutical Sciences, 2016, 5(3), 975-982.
12. Nataraj GD., Natesan SK., Amirtharaj V., Balakrishna A., 2016. Analytical Method Development & Validation of Amlodipine-An antihypertensive & Chlorthalidone-A thiazide diuretic By RP-UPLC Method, International Journal of Chemical & Pharmaceutical Sciences, 7(1), 12-17.
13. ICH, Q2A Text On Validation Of Analytical Procedures International Conference On Harmonization, Geneva, Oct, 1994,1-5.
14. ICH Q2B, Validation of Analytical Procedures, Methodology, International conference Harmonization, Genva, Nov,1996, 1-8.
15. Instruction manual model HPLC 2080 pump, Jasko Corporation.