



A REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF CHLORTHALIDONE IN PHARMACEUTICAL FORMULATION

BRIJESH SINGH^{*1}, D.K.PATEL² AND S.K.GHOSH¹

¹ Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India.

² Analytical Chemistry division, Indian Institute of Toxicology Research, M.G. Marg, Post Box No.-80, Lucknow, India.

Received – 7th May, 2009, Revised and Accepted – 5th July 2009

ABSTRACT

A simple, specific, sensitive, precise, and accurate high performance liquid chromatography method was developed for the determination of chlorthalidone in pharmaceutical tablet forms. Chlorthalidone is a diuretic drug frequently prescribed by Indian physicians for treatment of cardiac, hepatic, renal and pulmonary diseases. The method was carried out on reverse phase C-18 column (lichrospher, Merck®) (250×4mm, 5µm particle size) using a mixture of 50mM disodium hydrogen phosphate: methanol: acetonitrile in the ratio of 70:30:05 (pH adjusted to 3.5 with orthophosphoric acid) as mobile phase. Hydrochlorothiazide was used as internal standard. The detection was carried out by UV-detector at 220 nm at column temperature 30±2⁰C. The calibration curve was found to be linear in the range of 0.1 to 3.2 µg/ml. The intra-day and inter day percentage coefficient of variation was found 3.3085 and 0.3702 respectively.

Key words: Diuretic drug, chlorthalidone, HPLC, Pharmaceutical Formulation.

INTRODUCTION

Chlorthalidone is diuretic drug which increases the rate of urine flow. However diuretics also increase the rate of excretion of Na⁺ and accompanying anion Cl⁻. Sodium Chloride content in the body is the major determinant of the extracellular fluid volume, and most clinical applications of diuretics are directed towards reducing the extracellular fluid volume by decreasing the total body NaCl¹.

Diuretics are used, either alone or in combination with other drugs for the treatment of hypertension². Particularly diuretic compounds promote excretion of water and electrolytes via kidneys. They are also used in the treatment of heart disorder, hepatic, renal and pulmonary diseases when salt and water retention capacity results in oedema or ascites³.

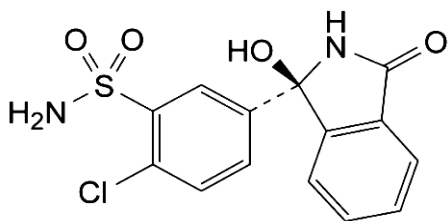
Chemically Chlorthalidone is 2-chloro-5-[(1*RS*)-1-hydroxy-3-oxo-2, 3-dihydro- 1*H*-

isoindol-1-yl] benzenesulphonamide⁴.

Chemical structure of chlorthalidone is shown below⁶.

Diuretics have been misused and abused in sports where weight categories are involved, such as weight-lifting, boxing and wrestling, in order to reduce body weight rapidly. Not only for ethical reasons but also because of serious health risks, the use of such type of compounds is prohibited by the International Olympic Committee⁵.

Literature survey reveals the reports of spectrophotometric and HPLC methods for the determination of chlorthalidone in pharmaceutical dosage forms and biological fluids^{4,6-2}. The objective of the present work was to develop a simple, efficient and reproducible method for quantitative determination of chlorthalidone in pharmaceutical preparations.



Chlorthalidone

Chemical structures of chlorthalidone

MATERIALS AND METHODS

Reagents and chemicals

All solvents were of HPLC grade and reagents were analytical grade. Acetonitrile and methanol were obtained from Merck[®], ortho phosphoric acid and disodium hydrogen phosphate were purchased from Ranbaxy. Water was purified with Milli-Q Millipore system. All the solvents and solution were filtered through membrane filter (Millipore millex[®]-FH, filter units, 0.22µm pore size) and degassed before use.

Chlorthalidone (assigned purity, 100.0%) was gift sample from M/s Ipca Laboratories, Mumbai, India, whereas hydrochlorothiazide (internal standard, assigned purity 99.68%) was from M/s Aristo Pharmaceuticals Pvt. Ltd. Mandideep, Raisen (M.P.) India. Commercially available chlorthalidone tablets were procured from the local market.

Instrumentation

Quantitative analysis was performed on isocratic high performance liquid chromatography system (HPLC, Waters, Milford, USA) with two Waters 515 pumps, a fixed wavelength programmable 2487 dual λ absorbance detector (Waters, Milford, USA). A guard column (C-18, shim-pack) (Merck, Germany), reverse phase C-18 column (lichrospher[®] Merck, 250×4 mm, 5µm particle size) and rheodyne injection valve

with 20 µl loop was used. The HPLC system was equipped with software Millenium³² (Waters, Milford, USA).

HPLC conditions

The eluting mobile phase was a mixture of aqueous disodium hydrogen phosphate (50 mM), methanol and acetonitrile in the ratio of 70:30:05. pH of the mobile phase was adjusted to 3.5 with ortho-phosphoric acid. Flow rate was maintained 1.0 ml/min. The UV detection was made at 220 nm and all analyses were done at column temperature (30 ± 2°C) under isocratic conditions.

Standard solution preparation

A stock solution of the drug and internal standard was prepared by dissolving 25 mg chlorthalidone and hydrochlorothiazide in 25 ml volumetric flask containing 15 ml mobile phase and warmed on water bath for about 15 min at 50° C. Content were shaken well, cool at room temperature and final volume was raised using mobile phase. Daily working standard solutions of chlorthalidone and hydrochlorothiazide were prepared by suitable dilution of stock solution with mobile phase.

Sample preparation

Twenty tablets (12.5 mg and 25 mg of each) were weighed and crushed to a fine powder. Powdered sample equivalent to 25 mg was transferred to 25 ml volumetric flask separately and contents were dissolved in 15 ml mobile phase. This mixture was shaken well on mechanical shaker for 20 min then warmed on water bath for 15 min and extraction was done 3 times with mobile phase then final volume was raised up to 25 ml. Finally the solution was filtered through 0.22 micron filter, filtered solution was accordingly diluted with mobile phase.

Calibration curves

Six sets of standard solution were prepared in mobile phase containing chlorthalidone at a concentration of 0.1, 0.2, 0.4, 0.8, 1.6 or 3.2 µg/ml (ppm) along with a fixed concentration of hydrochlorothiazide as internal standard. Each of these drug solutions (20 µl) was injected three times into the chromatographic system. The peak

area and retention time was recorded, mean value of peak areas were plotted against different concentrations.

RESULTS AND DISCUSSION

The present method was developed to quantify chlorthalidone in 12.5 mg and 25 mg tablets. Results are given in table 1. The drug content was analyzed and found to be 97.695 % of amount claimed on the average.

Table 1: Assay of chlorthalidone by the proposed HPLC method

Labeled amount (mg/tablet)	Observed amount (mg/tablet)	% Purity
12.5	12.323	98.581
12.5	12.013	96.175
25.0	24.582	98.328

The proposed method is simple and less time consuming for sample preparation and method was statistically proved for their accuracy and precision. Chromatogram of standards, blank, comparative standards with blank, formulation with internal standard are given in figures 1, 2, 3 and 4 respectively. No

interfering peaks were found in the chromatograms, indicating that the tablet excipients did not interfere with the estimation of drug by proposed HPLC method. The retention time (RT) of Internal Standard and chlorthalidone were found 3.34 and 10.82 min respectively.

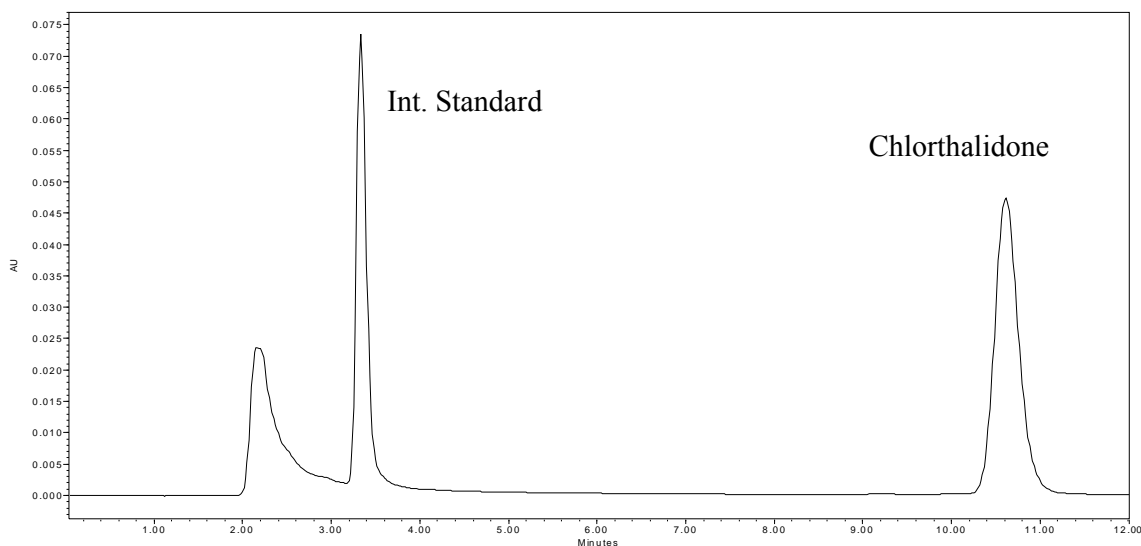


Fig. 1: A typical chromatogram of chlorthalidone and internal standard.

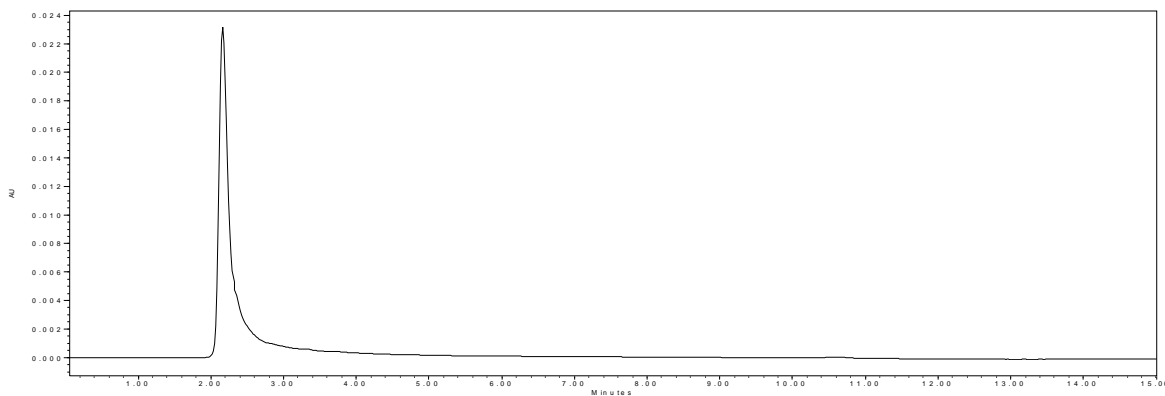


Fig. 2: Chromatogram of blank.

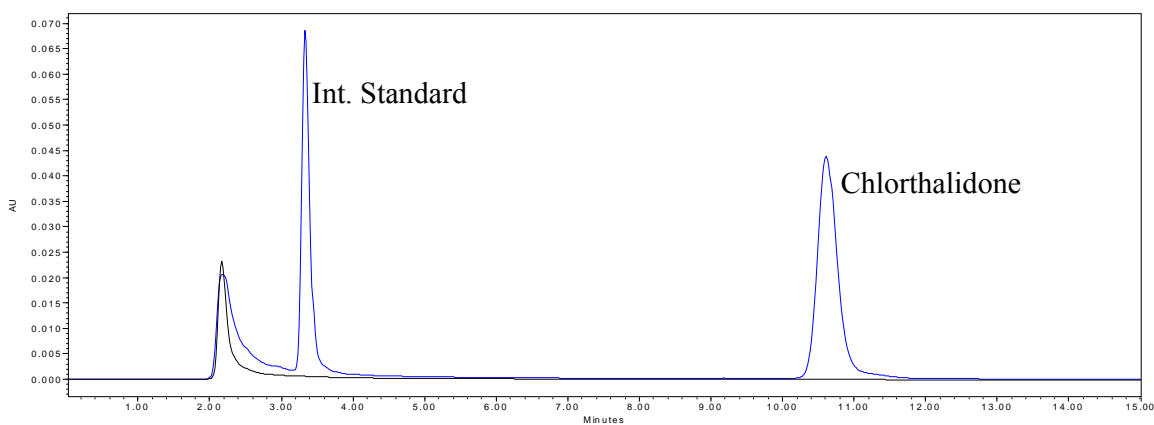


Fig. 3 : Comparative chromatogram of standard with blank

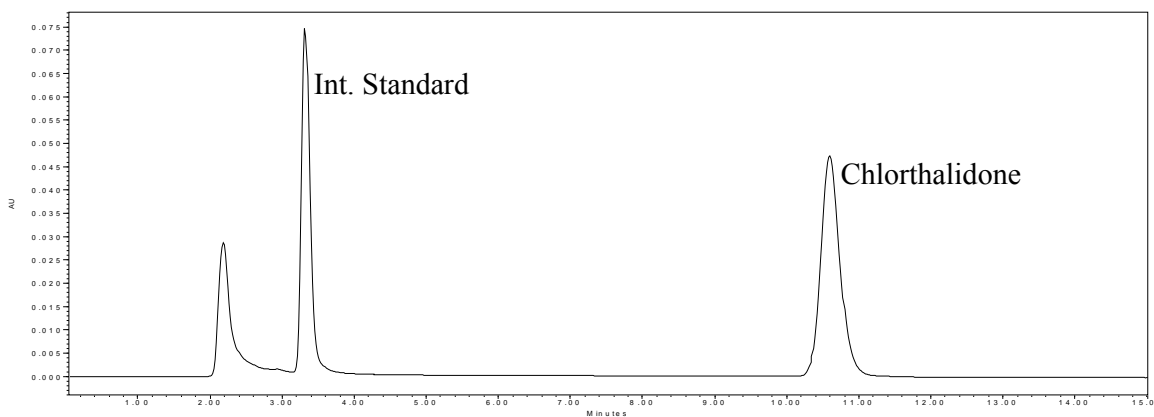


Fig. 4 : Chromatogram of formulation of chlorthalidone and internal standard

The calibration curve showed linearity over a concentration range from 0.1 to 3.2 $\mu\text{g/ml}$. It was found linear with a correlation coefficient

(r) of 0.9945, the representative linear regression equation being $Y = 0.7643X + 0.0945$ (fig. 5).

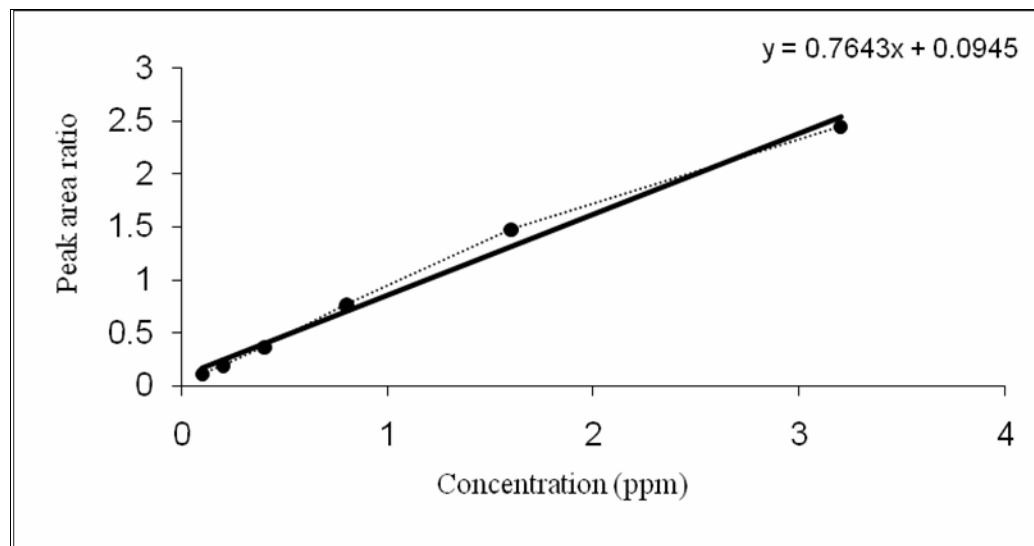


Fig. 5 : Calibration curve of chlorthalidone

Recovery test was performed in triplicate and average recovery was found 99.62 %, indicating that the proposed method for the determination of chlorthalidone in tablets is highly accurate (table- 2).

Table 2 : Recovery data of standard solutions added to the samples analyzed by using the proposed HPLC method

Amount of drug added (μg) to powdered tablet formulation	Amount (μg) found (n=3)	% Recovery (n=3)
0.25	0.256	102.20
0.50	0.511	102.16
0.75	0.708	94.49

Best resolution was found at pH 3.5, with eluting solvents 50mM Na_2HPO_4 : methanol: acetonitrile (in ratio 70: 30: 05) and flow rate 1.0 ml/min, after several trials with different pH (3.0-6.5) & with different proportion of eluting solvents. Inter and intra-day percentage coefficient of variation for assay of drug in pharmaceutical dosage form by proposed method was found to be 0.3702 and 3.308 respectively (table-3).

Table 3 : Inter and intra-day percentage coefficient of variation for chlorthalidone assay in pharmaceutical dosage forms by the proposed HPLC method

Concentration of Chlorthalidone in ($\mu\text{g}/\text{ml}$)	Observed concentration of Chlorthalidone (% coefficient of variation)	
	Intra-day	Inter-day
10.0	3.308	0.3702

In order to develop an efficient method for analysis of drug in pharmaceutical formulation, preliminary tests were performed with the objective to select adequate and optimum conditions. Parameters such as detection wavelength, mobile phase & their proportions, optimum pH and concentration of the standard solutions were exhaustively studied. The results of study showed that the proposed RP-HPLC method is simple, precise and accurate. It will be useful for the determination of chlorthalidone in its pharmaceutical dosage forms.

ACKNOWLEDGEMENTS

The authors acknowledge the Director IITR, Lucknow for providing all the facilities to carry out this work. The authors greatly acknowledge M/S IPCA Laboratories, Mumbai, India and M/S Aristo Pharmaceuticals Pvt. Ltd. Mandideep Raisen (M.P.) India for providing the gift samples of Chlorthalidone and Hydrochlorothiazide respectively.

REFERENCES

1. Goodman and Gilman's, The Pharmacological Basis of Therapeutics, 10th ed. (Eds. J. G. Hardman, and L. E. Limbird), McGraw Hill, London, 2001.
2. Avery's Drug Treatment, 4th Ed., Speight, T.M., Holford, N.H.G., Eds.; Adis International: Auckland, 1997.
3. Martindale, the Complete Drug Reference, 33rd ed. (Ed. S. C. Sweetman), Pharmaceutical Press, London, 2002.
4. British Pharmacopoeia, Published by The Stationery office, London, on behalf of Medicines and Healthcare Products Regulatory Agency (MHRA), 2007, vol.1 pp.484-485, vol.3 pp.2421.
5. Encyclopedia of Sports Medicine (Eds. A. Dirix, H.G.Knuttggen and Tittel), Vol. 1, International Olympic Committee, Blackwell, Oxford, 1988.
6. United States Pharmacopoeia, the National Formulary, Twinbrook Parkway, Rockville, MD 20852, 2003, pp. 440-441.
7. Rosenberg M. J., Lam K. K. and Dorsey T. E., Analysis of Chlorthalidone in Whole Blood by high performance liquid chromatography, *J. Chromatogr.* 375, 1986, 438-443.
8. Giachetti C., Tenconi A., Canali S. and Zanello G., Simultaneous determination of atenolol and chlorthalidone in plasma by high performance liquid chromatography. Application to pharmacokinetic Studies in man, *J. Chromatography. B* 698, 1997, 187-194.
9. Domingo E. Bonet, Hernfindez M. J. Medina, Ramos G. Ramis and M. C. Garcia Alvarez-Coque, high performance liquid chromatographic determination of diuretics in urine by micellar liquid chromatography, *J. Chromatogr.* 582, 1992, 189-194.
10. Carda-Broch, J. R. Torres-Lapasio, J. S. Esteve-Romero and M. C. Garcia-Alvarez-Coque, Use of three-factor interpretive optimization strategy in the development of an isocratic chromatographic procedure for the screening of diuretics in urine samples using micellar mobile phases, *J. Chromatogr. A* 893, 2000, 321-337.
11. Cooper S. F., Masse R. and Dugal R., Comprehensive screening procedure for diuretics in urine by high performance liquid chromatography, *J. Chromatogr.* 489, 1989, 65-88.
12. Tsai F. Y., Lui L. F. and Chang B., Analysis of diuretic doping agents by HPLC screening and GC-MSD confirmation, *J. Pharm. Biomed. Anal.* 9, 1991, 1069-1076.