



JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Development and Validation of UV- Spectrophotometric Method for Simultaneous Estimation of Spironolactone and Hydrochlorothiazide in Pharmaceutical Formulation

Z. M. Sayyed*, S. A. Shinde, V. J. Chaware, B. P. Chaudhari

Department of Quality Assurance, Anuradha College of Pharmacy, Chikhli. Dist-Buldana, Maharashtra, India-443201

ABSTRACT:

Simple, sensitive, specific and economic spectrophotometric method was developed and validated for simultaneous quantitation of Spironolactone (SPI) and Hydrochlorothiazide (HCTZ) in tablet dosage form. New method based on the simultaneous estimation of drugs in a binary mixture without previous separation was developed. In simultaneous equation method, Hydrochlorothiazide and Spironolactone were quantified using their absorptivity values of at selected wavelengths, viz., 273 nm and 238 nm respectively. The accuracy and reproducibility of the proposed method was statistically validated by recovery studies. The simultaneous equation method permits simple, rapid and direct determination of Hydrochlorothiazide and Spironolactone in commercially available tablet dosage form without previous separations and can therefore be used for routine analysis of both drugs in quality control laboratories.

KEY WORDS: Simultaneous Equation Method, λ_{max} , Validation, Spironolactone, Hydrochlorothiazide

Article history:

Received 04 Aug 2015

Accepted 24 Aug 2015

Available online 01 Nov 2015

Citation:

Sayyed Z. M., Shinde S. A., Chaware V. J., Chaudhari B. P. Development and Validation of UV- Spectrophotometric Method for Simultaneous Estimation of Spironolactone and Hydrochlorothiazide in Pharmaceutical Formulation. *J Pharm Sci Bioscientific Res.* 2015 5(6) 590-593

*For Correspondence:

Mr. Zuber Maheboob Sayyed.

Department of Quality Assurance,

Anuradha College of Pharmacy, Chikhli.

Dist.- Buldana, Pin – 443201,

Maharashtra (India)

(www.jpsbr.org)

INTRODUCTION:

Spironolactone is a potassium-sparing diuretic used in the treatment of heart failure and ascites in patients with liver disease, low-renin hypertension, hypokalaemia and secondary hyper aldosteronism^[1,2]. Spironolactone is a specific antagonist for the mineralocorticoid receptors, thus it compete with aldosterone for its intracellular receptors, thereby reducing Na^+ , water retention and K^+ secretion, resulting in increase in the volume of tubular fluid^[3]. Spironolactone chemically is 7 α -acetyl thio-3-oxo-17 α -pregn-4-ene-21, 17 β -carbolactone-4-chloro-N-furfuryl-5-sulphamoyl anthranilic acid with a molecular formula of $\text{C}_{24}\text{H}_{32}\text{O}_4\text{S}$ and molecular weight of 416.574 g/mol^[4-6]. It is an official drug in Indian Pharmacopoeia and British Pharmacopoeia (Fig. No. 1-A).

Hydrochlorothiazide, 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-Sulphonamide 1, 1- dioxide, is a diuretic, which inhibits active chloride reabsorption at the early distal tubule via the Na-Cl co-transporter, resulting in an increase in the excretion of sodium, chloride, and water^[7-10]. It has been used in the treatment of several disorders including edema, hypertension, diabetes insipidus, and hypoparathyroidism^[11]. Hydrochlorothiazide having molecular formula $\text{C}_7\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2$ and molecular weight 297.739 g/mol. It is official in IP, BP and USP^[12-15]

(Fig. No. 1-B).

To our knowledge simple and economical analytical method for simultaneous determination of Spironolactone and Hydrochlorothiazide has not been reported so far. The present communication describes simple, sensitive, accurate, rapid and economic method for Simultaneous estimation of Spironolactone and Hydrochlorothiazide in tablet formulation. The developed methods were validated and found to be accurate, precise and reproducible.

MATERIALS AND METHODS

Instruments:

a) Spectrophotometer Double beam UV –Visible spectrophotometer with 1 cm matched quartz cell Model SL 218- Elico.

b) Electronic Balance Shimadzu.

Reagents and Chemicals:

Spironolactone and Hydrochlorothiazide reference standard were kindly provided by Mylan Pharmaceuticals Pvt. Ltd. 0.1 N Sodium Hydroxide of analytical grade was used as a solvent throughout the analysis.

Marketed Preparation:

The brand name of marketed tablet formulation is Aldactazide 25 containing Spironolactone 25 mg and Hydrochlorothiazide 25 mg.

Preparation of standard stock solution:

Spironolactone:-Standard stock solution of Spironolactone was prepared by dissolving 10 mg Spironolactone in 50 ml of 0.1N sodium Hydroxide and sonicates for 20 min and then dilute up to 100 ml to produce a concentration of 100 µg/ml. which is the standard stock solution.

Hydrochlorothiazide: - Standard stock solution of Hydrochlorothiazide was prepared by dissolving 10 mg of Hydrochlorothiazide in 100 ml of 0.1N sodium Hydroxide to produce a concentration of 100 µg/ml. which is the standard stock solution.

Determination of λ max of Spironolactone and Hydrochlorothiazide:

The aliquot portion of stock standard solutions of Spironolactone and Hydrochlorothiazide were diluted appropriately with 0.1 N sodium Hydroxide to obtain concentration 25µg/ml and 25µg/ml respectively. The solutions were scanned in the range of 400 – 200 nm in 1 cm cell against blank. The λ max was determined on double beam UV – Visible Spectrophotometer using 0.1 N sodium Hydroxide as blank. The λ max of Spironolactone and Hydrochlorothiazide was found to be 238 nm and 273 nm respectively. Determination of λ max of Spironolactone and Hydrochlorothiazide are shown in **Fig. No. 2.**

Preparation of Calibration Curve:

The aliquot portion of stock standard solutions of Spironolactone and Hydrochlorothiazide were diluted appropriately with 0.1 N Sodium Hydroxide as solvent to get a series of concentration between 0.25-1.25 µg/ml of Spironolactone and 6.0-30.0 µg/ml of Hydrochlorothiazide. The absorbance of each solution was measured at 238 nm and 273 nm in 1cm cell against 0.1 N sodium hydroxide as blank. The graphs plotted as concentration Vs absorbance at selected wavelengths for SPI and HCTZ are shown in **Fig. No. from 3 to 6.**

Determination of A (1%, 1 cm) of drugs at selected wavelengths :

Aliquot portions of SPI from stock solution were transferred to five 10 ml volumetric flasks; volume was adjusted to mark to obtain the concentration of 25 µg/ml. Similarly, aliquot portions from HCTZ stock solution was transferred to 10 ml volumetric flasks; volume was adjusted to mark to obtain concentration of 25 µg/ml. Absorbance of these solutions was recorded at two wavelength 238 nm and 273 nm. A (1%, 1 cm) values of drugs were calculated using following formula-

$$A (1\%, 1 \text{ cm}) = \text{Absorbance} / \text{Concentration (g / 100 ml)}$$

Results of A (1 %, 1 cm) of drugs are given in **Table No. 1 and 2.**

Simultaneous Estimation of drugs in tablets :

Twenty tablets each containing 25 mg of SPI and 25 mg of HCTZ were weighed and average weight was calculated, ground to fine powder. An accurately weighed quantity equivalent to 25 mg of tablet powder

was transferred to 100 ml volumetric flask containing about 40 ml 0.1 N sodium Hydroxide, sonicated for 10 min and volume was made up to the mark with the same solvent to get the concentration of 25 µg/ml SPI and 25 µg/ml of HCTZ and filtered through Whatman filter paper (No. 41). The absorbances were recorded. The concentrations of two drugs in sample were determined using equation No. (1) and (2) results are given in **Table No. 3**.

Simultaneous Equation Method

$$C_x = \frac{A_{2y1} - A_{1y2}}{a_{x2y1} - a_{x1y2}} \text{----- (1)}$$

$$C_y = \frac{A_{1x2} - A_{2x1}}{a_{x2y1} - a_{x1y2}} \text{----- (2)}$$

Where,

A₁ and A₂ are absorbance of sample solution at 238 nm and 273 nm respectively,

a_{x1} and a_{x2} are the absorptivities of SPI at 238 nm and 273 nm respectively and

a_{y1}, a_{y2} are the absorptivities of HCTZ at 238 nm and 273 nm respectively.

C_x and C_y are the concentrations of SPI and HCTZ in µg/ml respectively.

Method Validation:

The method validation parameters like linearity and accuracy were checked as per ICH (International conference on Harmonization) guidelines.

Linearity: The linearity for SPI and HCTZ were determined at five concentration level, ranging from 0.25-1.25 µg/ml and 6-30 µg/ml respectively using working standards.

Accuracy: The accuracy of the method was determined by recovery studies. The recovery studies were performed by the standard addition method and the percentage recoveries were calculated.

Precision: The precision of the method was evaluated by inter day and intraday variation studies. In intraday studies, working solutions of standard and sample were analysed thrice in a day and percentage relative standard deviation (% RSD) was calculated. In the inter day variation studies, working solution of standard and sample were analysed on three consecutive days and percentage relative standard deviation (% RSD) was calculated.

Repeatability: Repeatability was determined by analyzing SPI (25 µg/ml) and HCTZ (25 µg/ml) of drug solutions for five times.

Limit of Detection and Limit of Quantitation: The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula.

$$LOD = 3.3 (\sigma / S)$$

Where, S = slope of calibration curve, σ = standard deviation of the response.

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula.

$$LOQ = 10 (\sigma / S)$$

Where, S = slope of calibration curve, σ = standard deviation of the response.

The results of Validation Parameters are given in **Table No.4**.

RESULTS AND DISCUSSION

The wavelength of Spironolactone and Hydrochlorothiazide were found to be 238 nm and 273 nm respectively in the solution of 0.1 N Sodium Hydroxide. In this method drugs obey Beer's law in the concentration range of 0.25-1.25 µg/ml of SPI and 6.0-30.0 µg/ml of HCTZ. The results showed an excellent correlation between absorbance's and concentration of the drugs. Standard calibration curves for SPI and HCTZ were linear with correlation coefficients (r²) values in the range of 0.991- 0.999 at all the selected wavelengths. The results of analysis of the marketed formulation by Simultaneous Equation Method are shown in **Table No.3**. Method validations were done as per ICH Validation parameters like Accuracy, Precision, Linearity range, Repeatability, Limit of Detection (LOD) and Limit of Quantitation (LOQ). The method showed accuracy in the range of 98.95-99.15%. Summary of Validation parameters are shown in **Table No. 4**.

CONCLUSION

Literature review indicated that, various methods have been reported for the analysis of Spironolactone and Hydrochlorothiazide in formulation by RP-HPLC, HPTLC. But no analytical methods were reported for the

estimation of these drugs using UV- spectrophotometric method. Simple UV spectrophotometric method was developed for the simultaneous determination of Spironolactone and Hydrochlorothiazide in tablet formulation. To the best of our knowledge, the present study is the first report for the purpose. The present method has been validating for Spironolactone and Hydrochlorothiazide as per ICH guidelines. It can therefore be concluded that use of these methods can save much time and money and they can be with accuracy.

ACKNOWLEDGEMENT

The authors wish to thank Mylan Pharmaceuticals Pvt. Ltd. For providing gift samples of Spironolactone and Hydrochlorothiazide.

REFERENCES

- Vadalia KR, Chaudhary A, and Thummer P. Ratio Derivative Spectrophotometric Method for the Simultaneous Estimation of Metolazone (METO) and Spironolactone, IJPSR, 2012; 3(10): 3999-03.
- Parimoo P, Bharathi A and Padma K. Simultaneous Determination of Spironolactone with Hydroflumethiazide and Spironolactone with Frusemide in Combination formulations by UV- Absorption Method, Indian J.Pharm. Sci., 1995; 57(3): 126-29.
- Maha A, Abdelkawy M, and Nada S. Stability Indicating Chromatographic Method for Determination of Hydrochlorothiazide and Spironolactone in Pharmaceutical Formulation in Presence of Impurities and Degradants, Journal of Chromatographic Science, 2011; 49:129-35.
- Luis ML, Garcia JM, Jimenez F, Jimenez AI, and Arias JJ, Simultaneous Estimation of Chlorthalidone and Spironolactone with Univariate and Multivariate Calibration, Wavelength Range Selection, J. AOAC. Int., 1999; 82(5): 1054-63.
- Millership JS. Ratio Spectra Derivative Spectrophotometry for the Determination of Furosemide and Spironolactone in a Capsule Formulation, Farmaco, 2005; 60: 333-38.
- Dinc E and Ustundag O. Spectrophotometric Quantitative Resolution of Hydrochlorothiazide and Spironolactone in Tablets by Chemometric Analysis Methods, Farmaco, 2003; 58: 1151-61.
- Singh S, Yadav AK and Gautam H. Simultaneous Estimation of Valsartan and Hydrochlorothiazide in Solid Dosage Form Using UV Spectroscopy, Bulletin of Pharmaceutical Research, 2011; 1(3):10-12.
- Bhatia NM, Desai BR, and Jadhav SD. Simultaneous Estimation of Losartan Potassium and Hydrochlorothiazide from Tablet by First order Derivative Spectroscopy, Int J Pharm. Sci, 2012; 5(1): 464-66.
- Mhaske RA, Garole DJ, and Sahasrabudhe S. RP-HPLC Method For Simultaneous Determination of Amlodipine Besylate, Valsartan, Telmisartan, Hydrochlorothiazide and Chlorthalidone: Application to Commercially Available Drug Products, IJPSR, 2012; 3(1): 141- 49.
- Meltze EL, Eichner GL, and Ural JE. A comparison of Hydrochlorothiazide and Chlorothiazide in the treatment of hypertension, Am. J. Cardiol, 1959; 4(6): 741-44.
- Bhusari VK, Sunil R and Dhaneshwar SR. Validated HPTLC Method for simultaneous Estimation of Atenolol, Hydrochlorothiazide and Amlodipine Besylate in Bulk drug and Formulation, International Journal of Analytical and Bioanalytical Chemistry 2011; 1(3): 70-76.
- Safeer K, Anbarasi B and Kumar SN. Analytical Method Development and Validation of Amlodipine and Hydrochlorothiazide in Combined Dosage form by RP-HPLC, International Journal of Chem. Tech Research, 2010.; 2(1):21-25.
- Carlucci G and Palumbo G. Simultaneous Determination of Losartan and Hydrochlorothiazide in Tablets by High Performance Liquid Chromatography, J. Pharm. Biomed. Anal, 2000; 23(1): 185-90.
- Alaa EG and Ahmed A. Application of LC and HPTLC Densitometry for the Simultaneous Determination of Benazepril Hydrochloride and Hydrochlorothiazide, J. Pharma. Biomed. Anal, 2001; 25(2):171-179.
- ICH Steering Committee. ICH Q2B Validation of Analytical Procedures: (1996) methodology. London (CPMP/ICH/281/95): European Agency for the Evaluation of Medicinal Products, International Commission on Harmonization, Switzerland.



Journal of
Pharmaceutical Science and
Bioscientific Research Publication

www.jpsbr.org
jpsbronline@rediffmail.com
Copyrights 2011 JPSBR Publication Allrights Reserved