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SPECTROPHOTOMETRIC QUANTITATIVE ESTIMATION OF HYDROCHOLOROTHIAZIDE AND LISINOPRIL IN BULK DRUGS AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, accurate, precise, economical and reproducible UV Spectrophotometric method has been developed for the simultaneous estimation of Hydrochlorothiazide and Lisinopril in bulk and in marketed combined tablet dosage form. The stock solutions were prepared in pH 1.2 buffer followed by further required dilutions with pH 1.2 buffer. Vierordt's simultaneous equation method was developed and is validated statistically as per ICH guidelines. The absorbance maxima of Hydrochlorothiazide and Lisinopril were found to be 269.8nm & 209.4nm respectively. Linearity was observed by linear regression equation method for both drugs in different concentration range. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. The % R.S.D. were found to be less than 2 % as required by ICH guidelines, which indicates the validity of methods. Statistical analysis proves that the proposed method can be effectively applied for the simultaneous estimation of these two drugs in bulk & combined dosage forms.

KEYWORDS: Hydrochlorothiazide, Lisinopril, Vierordt's simultaneous equation method, etc.

INTRODUCTION

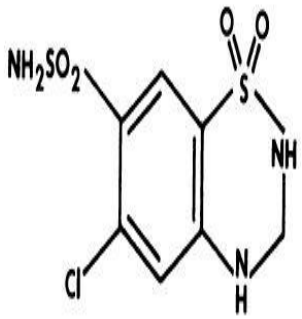
Hydrochlorothiazide, inhibitors of angiotensin-converting enzyme (ACE) are widely used for the treatment of mild to moderate hypertension and heart failure either alone or in

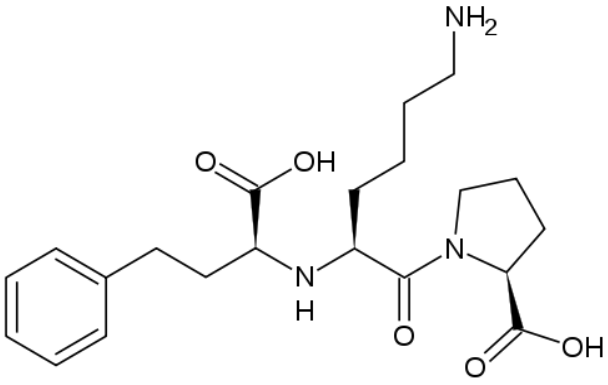
conjunction with hydrochlorothiazide, a thiazide diuretic.[1] Hydrochlorothiazide increases the rate of urine excretion by the kidneys, primarily through decreased tubular reabsorption of sodium and chloride and by increased osmotic transport of water to the renal tubules. Thiazide diuretics are extremely useful in the treatment of oedema associated with mild to moderate congestive heart failure. Moreover, these diuretics are also primary agents used in the control of hypertension, either alone or in combination with other drugs such as ACE in Inhibitors. Their hypotensive effect is believed to be due initially to the reduction of blood volume by Na+ depletion, and later by direct relaxation of arteriolar smooth muscle.[2,3,4]

Lisinopril.[5] is the lysine-analog of enalapril (ACE).Which converts angiotensin I to angiotensin II as angiotensin II is a vasoconstrictor and a negative feedback mediator for renin activity, lower angiotensin II plasma levels result in decreased blood pressure and increase in plasma renin activity. Baroreceptor reflex mechanisms, stimulated by the fall in blood pressure, release kinases II, an enzymes identical to ACE that degrades bradykinin, a vasodilator.[6,7]

In the literature survey, Different spectrophotometric and HPTLC-densitometry methods are presented for the simultaneous determination of Lisinopril and Hydrochlorothiazide in pharmaceutical tablets by using chloroform–ethyl acetate–acetic acid (10:3:2 by vol.) as mobile phase.[8]

Extensive literature survey reveals that no Spectrophotometric method is available for simultaneous determination of HCZ and LP in combination in HCl buffer of pH 1.2. Aim of present work was to develop simple, precise, accurate and economical Spectrophotometric methods for simultaneous determination of binary drug formulation in biological fluids at three different pH. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines.[9]





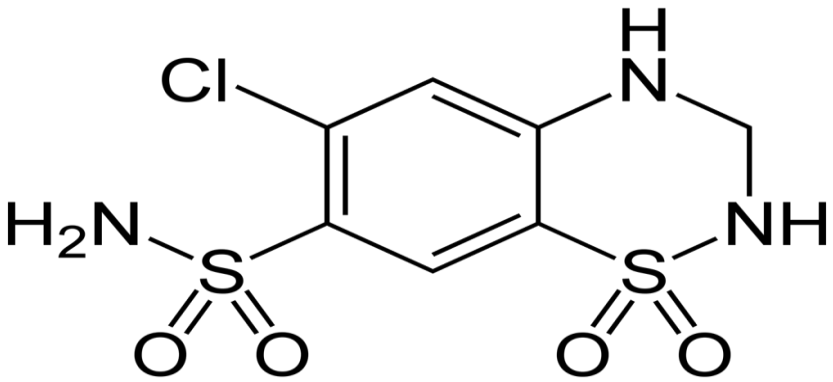


Figure 1 Structural formula of Hydrochlorothiazide. Figure 2 Structural Formula of Lisinopril.

MATERIALS AND METHODS

Instruments

Quantitative estimation was performed on Shimadzu UV 1700 double beam UV-Visible spectrophotometer with matched 1 cm path-length quartz cells. Absorption spectra was recorded on a medium scan speed, setting slit width to be 1 nm and sampling interval to be auto. pH meter (Labindia) was used.

Chemicals and Reagents

HCZ and LP obtained from Yarrow Chem. Pvt. Ltd. A commercial sample HCZ and LP tablets were procured from local market and used within their shelf-life period. Potassium Chloride and Hydrochloric acid was procured from Loba Chemie Pvt. Ltd., Mumbai and S.D. Fine Chemical Limited, India respectively. To prepare HCl Buffer of pH 1.250ml of 0.2M KCl was taken in 200ml volumetric flask. 85ml of 0.2M HCl was added to the volumetric flask. This solution was diluted to 200ml with distilled water.

Vierordt's simultaneous equation method[10-12]

This method of analysis is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. The quantification analyses of HCZ (X) and LP (Y) in a binary mixture were performed with the following equations:

$$C_x = (A_2a_{y1} - A_1a_{y2}) / (a_{x2}a_{y1} - a_{x1}a_{y2}) \quad \text{…… (Eqn. 1)}$$

$$C_y = (A_1a_{x2} - A_2a_{x1}) / (a_{x2}a_{y1} - a_{x1}a_{y2}) \quad \text{…… (Eqn. 2)}$$

Where -

C_x and C_y are the concentration of X and Y, respectively in the diluted sample. a_{x1} and a_{x2} are absorptivities of X at λ_1 and λ_2.

a_{y1} and a_{y2} are absorptivities of Y at λ_1 and λ_2.

Preparation of Standard Stock solution

Standard stock solution of 100 µg/ml of HCZ and LP were prepared by dissolving accurately weighed quantity (10mg) of each drug in 100 ml HCl buffer of pH 1.2. The aliquots of standard stock solution of drugs were diluted separately with pH 1.2 buffer to obtain working standard solutions with final concentration of 10 µg/ml of each drug and each working standard solution was scanned between 200-380 nm in Shimadzu UV visible spectrophotometer. Overlain absorption spectrum of both drugs was recorded and is depicted in Figure 3. The spectra exhibit major absorbance maxima at 269.8nm and 209.4nm for HCZ and LP, respectively which revealed that the peaks are well satisfying the criteria for obtaining maximum precision based on AT and LP, respectively.

For the preparation of calibration curve of HCZ and LP at selected two wavelengths; the standard solution (100 µg/ml) was further diluted with HCl buffer of pH 1.2 to obtain different concentration range for HCZ and LP and the absorbance was measured at 269.8nm and 209.4nm for both drugs and calculated the absorptivity.

Analysis of Tablet Formulations

For analysis of commercial formulations of tablets, 20 tablets were weighed, powdered and accurately weighed and finely powdered. The amount of powder equivalent to 50 mg of HCZ and 50 mg of LP were weighed and transferred into 100 ml volumetric flask. Methanol (10 mL) was added to it and sonicated for 10 minutes and volume was made up to mark with pH 1.2 buffer. The solution was filtered through Whatman filter paper No. 41. From the above solution suitable aliquot was diluted with pH 1.2 buffer to get concentration 10 µg/mL of each drug. The absorbance of sample solutions were measured at both selected

wavelengths.

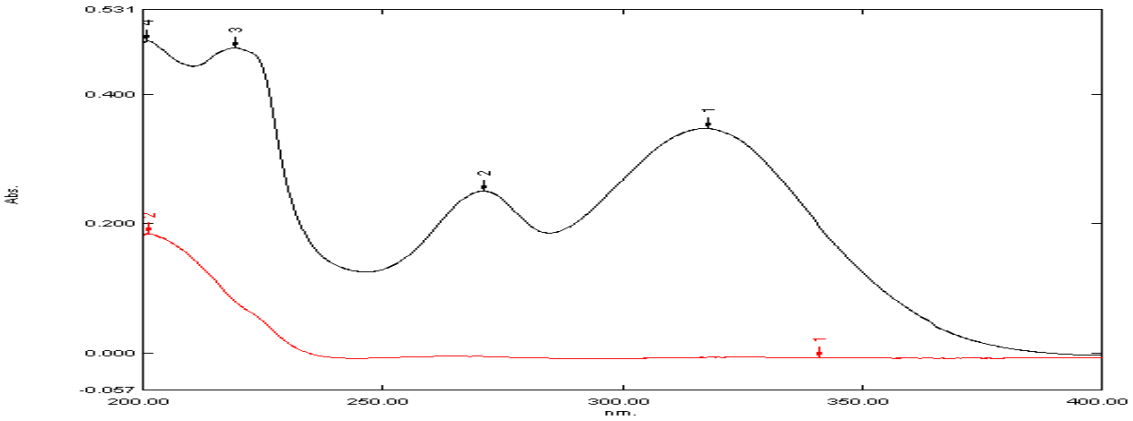


Figure 03 Overlain Absorption Spectra of HCZ and LP in pH 1.2 buffer.

Validation of proposed method[11]

The method was validated according to ICH guidelines for validation of analytical procedure in order to determine sensitivity, linearity, precision, and accuracy.

Linearity

The linearity of measurement was evaluated by analyzing different concentration of standard solution of both drugs at the λ_{max} of HCZ i.e. 269.8nm and at λ_{max} of LP i.e. 209.4 nm. The response was plotted against concentration of the analyte. Linearity of the calibration curve was demonstrated by applying least square regression analysis to the plot obtained.

Accuracy

To ascertain accuracy of the proposed method, different levels of drug concentrations (LQC, MQC and HQC) were prepared from independent stock solution and analyzed. Accuracy was assessed as the mean percentage Bias.

Precision

Precision was studied to find out intra-day and inter-day variations in the proposed method. Different levels of prepared drug concentrations were run in triplicate in the same day (intra-day variation) and for three consecutive days (inter-day variation). % Relative standard deviation (% RSD) were calculated which should be less than 2%.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD was determined using the relation 3.3 σ/s where 'σ' is the standard deviation of the

response and 's' is the slope of the calibration curve. The standard deviation of the response can be obtained either by measuring the standard deviation of the blank response or by calculating the residual standard deviation of the regression line or by calculating the standard deviation of the y-intercept of the regression line, i.e. the standard error of the estimate. Similarly, LOQ was determined using the relation 10 σ/s.

RESULT AND DISCUSSIONS

Simultaneous equation method was proposed as a suitable method for the analysis of drugs HCZ and LP in dosage forms. A series of standard solutions were prepared for HCZ and LP, and absorbance's of solutions were recorded at 269.8nm and 209.4nm to plot a calibration curve of absorbance versus concentration. The calibration curves were found to be linear in concentration range under study (Table-2). Regression equation and Absorptivity values of AB and TEL were determined at selected wavelengths are presented in Table- I.

Table 1 Result of Analytical method Development in 1.2 pH buffer

Parameter		**HCZ at 269.8nm**	**LP at 269.8nm**	**HCZ at 209.4nm**	**LP at 209.4nm**
Absorptivity		486.77	38.81	1948	388.18
Regression Equation	Slope	0.0496	0.039	0.1983	0.0393
	Intercept	0.0022	0.0013	0.0022	0.0012
	Correlation coefficient(r^2)	0.9995	0.9996	0.9995	0.9996

The concentration of two drugs in mixture was calculated by using following equations.

$$C_x = (A_2a_{y1} - A_1a_{y2}) / (a_{x2}a_{y1} - a_{x1}a_{y2}) \quad \text{…… (Eqn. 1)}$$

$$C_y = (A_1a_{x2} - A_2a_{x1}) / (a_{x2}a_{y1} - a_{x1}a_{y2}) \quad \text{…… (Eqn. 2)}$$

Where - C_x and C_y are the concentration of X and Y, respectively in the diluted sample.

The absorbance of the diluted samples at λ_1 (HCZ λ_{max}) are A1 **($A_1 = a_{x1}bc_x + a_{y1}bc_y$).**
The absorbance of the diluted samples at λ_2 (LP λ_{max}) are A_2 **($A_2 = a_{x2}bc_x + a_{y2}bc_y$)**.

a_{x1} and a_{y1} are the absorptivity's of Hydrochlorothiazide and Lisinopril respectively at λ_1 i.e. 269.8 nm. a_{x2} and a_{y2} are the absorptivity's of Hydrochlorothiazide and Lisinopril respectively at λ_2 i.e. 209.4 nm.

To prove the validity and applicability of the proposed method, studies were carried out as per ICH Guidelines and their results are stated in Table 3. Satisfactory results were obtained

with % Bias and %RSD value less than 2%; thus conforming the accuracy and precision of proposed method. LOD and LOQ values for AB and TEL are stated in Table 3.

Table 2 Validation of proposed method

Parameter		HCZ at 269.8nm	LP at 269.8 nm	HCZ at 209.4nm	LP at 209.4nm
Beer's Law Limit (µg/ml)		4-20	50-250	1-5	5-25
LOD (µg/ml)		0.2732	2.13	0.0732	0.4792
LOQ (µg/ml)		0.8278	6.44	0.222	1.452
Precision (%RSD)	Interday	0.75-1.17	0.53-0.22	0.093-0.068	0.36-0.021
	Intraday	0.70-1.18	0.56-0.41	0.059-0.038	0.11-0.6084
Accuracy (% Bias)		0.71-1.16	0.44-0.22	0.80-0.097	0.093-0.021

The percentage of purity of HCZ and LP in tablet dosage form is shown in Table 2. The precision of the spectrophotometer system was determined using the %RSD of the absorbance for six replicate injections of the drug. The %RSD was less than 2 indicating precision of the method. Precision data were present in Table 3.

Table 3 Determination of AB and TEL in combined tablet dosage form

Brand	Tablet Content	Label content (mg)	Amount found (mg)	%Amount*	± SD*	RSD (%)*
I	HCZ	12.5	12.19	99.38	± 0.065	0.155
	LP	10	9.81	99.62	± 0.048	0.174
II	HCZ	12.5	12.59	100.18	± 0.071	0.183
	LP	10	10.04	100.08	± 0.053	0.195

*Mean of six readings

CONCLUSION

A convenient and rapid UV method has been developed for simultaneous estimation of Hydrochlorothiazide and Lisinopril in available dosage form. The assay provides a linear response across a wide range of concentrations. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. Intra-day and inter-day % R.S.D. values were found to be less than 2 % as required by ICH guidelines, which indicates the validity of methods; hence, this method can be easily and conveniently adopted for routine analysis of Hydrochlorothiazide and Lisinopril in pure form and its dosage forms.

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REFERENCES

1. G.H. Cocolas, J.N. Delgado, W.A. Remers (Eds.), Textbook of Organic Medicinal and Pharmaceutical Chemistry, Lippincott-Raven, Philadelphia., 1998; 10: 603.
2. W.O. Foye, Principles of Medicinal Chemistry, 2nd ed, Henry Kimpton, London, 1981.
3. D.A. Koechel, J.N. Delgado, W.A. Remers (Eds.), Textbook of Organic Medicinal and Pharmaceutical Chemistry, Lippincott-Raven, Philadelphia., 1998; 10: 553.
4. Available from http://www.drugbank.ca/drugs/DB00999[Last accessed on 2015 August 9].
5. The American Society of Health-System Pharmacists http://www.drugs.com/monograph/lisinopril.html. [Last accessed on 2015 August 9].
6. Baker KM, Morgan HE. Cardiac hypertrophy. Mechanical, neural and endocrine dependence. Circulation., 1991; 83: 13–25.
7. Dunn FG, Olgman W, Ventura HD, Messerli FH, Kobrin I, Frohlich ED. Enalapril improves systemic and renal hemodynamics and allows regression of left ventricular mass in essential hypertension. Am J Cardiol., 1984; 53: 105–108.
8. Alaa El-Gindy a, Ahmed Ashour, Laila Abdel-Fattah, Marwan M. Shabana d, Journal of Pharmaceutical and Biomedical Analysis., 2001; 25: 923–931.
9. ICH, Q2 (R1), Harmonised tripartite guideline, Validation of analytical procedures: text and methodology International Conference on Harmonization ICH, Geneva, Nov 2005.
10. Tangri P, Arya M, Lakshmayya. International Journal of Pharmaceutical Research and Development., 2012; 4(3): 296-301.
11. Gangola R, Kaushik S, Sharma P. Spectrophotometric Simultaneous Determination of Hydrochlorothiazide and Telmisartan in Combined Dosage. Journal of Applied Pharmaceutical Science., 2011; 1(1): 46-49.
12. Nilesh Jain *et al*. Simultaneous Spectrophotometric Estimation of Lercanidipine Hydrochloride and Atenolol in Tablet Dosage Form. Eurasian J Anal Chem., 2011; 6(2): 84-90.