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A Novel Quantification Method for the Simultaneous Estimation of Losartan Potassium and Hydrochlorothiazide in Bulk and Tablet Dosage Form Using Simultaneous Equation Method

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ABSTRACT

In this present paper, a novel, sensitive, UV- Spectrophotometric simultaneous equation method was proposed for the estimation of Losartan Potassium (LOS.P) and Hydrochlorothiazide (HTZ) in bulk and tablet dosage form. Water: Methanol in the ratio of 95:5 v/v was used as a solvent which exhibits maximum absorption at 215nm for LOS.P and 272nm for HTZ. Beers law was obeyed over the linear range for LOS.P is 1-20µg/ml and HTZ. The correlation coefficient (R^2) was found 0.980 for LOS.P and 0.992 for HTZ respectively. The method was successfully applied for the assay of the both drugs in tablet with 99.2 % and 98.67 % purity for LOSP and HTZ respectively. The developed method was fully validated as per ICH guidelines. The percentage relative standard deviation (RSD) for accuracy was less than 2. The percentages RSD of the intra-day and inter-day variations were found also less than 2%. The limit of detection and quantification were 0.32µg/ml and 0.97 µg/ml for LOS.P and 0.09 µg/ml and 0.248 µg/ml for HTZ which indicates the sensitivity of the developed method. The present method also demonstrated robust on changing λ_{max} and solvent volume ratio up to ±2%. Empirical evidence from developed method concludes that it was simple, sensitive, reliable, and useful for the routine quality control analysis of LOS.P and HTZ in bulk and tablet dosage form.

Keywords: Losartan; hydrochlorothiazide; simultaneous equation, Spectrophotometry.

INTRODUCTION

chemically Losartan potassium is potassium[2-butyl-5chloro-3-[4-[2-(1,2,3-triaza-4-azanida cyclopenta- 2,5 diene –5yl) phenyl] imidazol-4-yl] methanol [1] belongs to angiotensin-II receptor antagonist, which lowers the blood pressure and improves blood flow [2-3] HTZ which is chemically benzothiadiazine derivatives, (shown in fig.1.) belongs to the category of thiazide diuretic along with diuretic medication it is often used to treat elevated blood pressure [4-5]. Combining form of LOS.P and HTZ is widely prescribing worldwide for the treatment of essential hypertension in patients whose blood pressure is not controllable on LOS.P or HTZ alone [6] and it is not also used in patients having renal impairment and hemodyalsis. Hence the proper analytical method for the quantification of both drugs in pharmaceutical dosage form is highly necessary. Literature survey reveals that there is an availability of Spectrophotometric [7-8] Capillary electrophoresis [9] HPLC with other combination [10] for LOS.P and HTZ individually, though both the drugs were official in Indian pharmacopeia separately but the analytical methods in combine dosage form is still not official in any pharmacopeia. However there is an availability of simultaneous determination of both analytes using HPLC[11-15] and very few spectrophotometric methods by using simultaneous

equation [16-17] states that either the initialization of fully organic solvents or higher organic solvent ratios for the preparation of standard and working solutions takes place. We also found ambiguity on the validation results after repetition of available methods so the question on the reliability of the available methods rises. It is a prime for an analyst to develop easy, stable, validated economic method for the analysis of API's as marketed formulation which will be acceptable and indirectly reduces the cost of marketed formulation. So that common people can be afforded easily [18]. Hence it is necessary to develop a easy, reliable and validated method for the above mentioned drugs based on ICH Guidelines [19]. Keeping the above facts in mind we planned to develop reliable and indirectly tried to make this method more sensitive by diminishing the LOD and LOQ limits which can minimize the cost of dosage form.

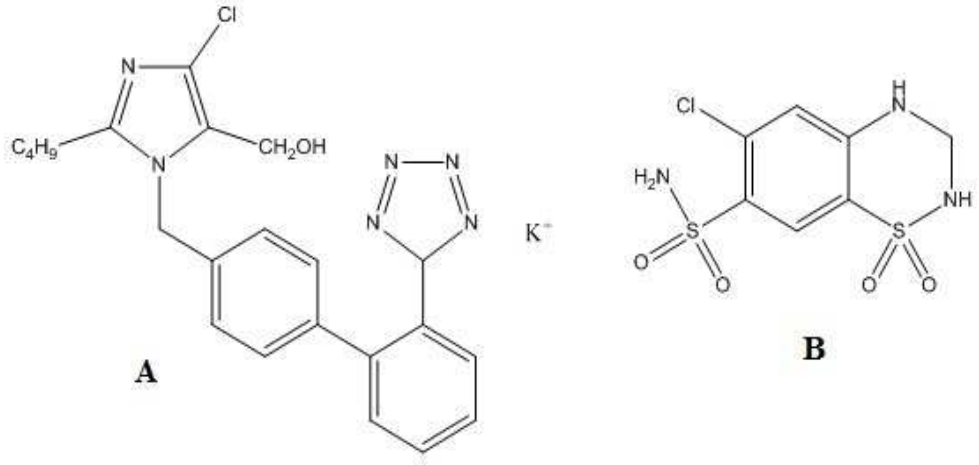

Fig.1. Chemical structure of Losartan Potassium (A) and Hydrochlothiazide (B)

MATERIALS AND METHODS

Instruments and reagents

Pure LOS.P and HTZ standards were received from Arabindo Pharma Limiteds Hyderabad as a gift sample LOS.P and HTZ tablets (Cosart-H – LOS.P-50µg and HTZ – 12.5µg) were purchased from local market, Hyderabad, India. Other reagents used for the study in analytical grade. Absorbance measurements were made using Analytical Technologies (T-60) Ultraviolet-Visible spectrophotometer provided with 1 cm quartz cells and temperature was controlled for all spectrophotometric measurements.

LOS.P and HTZ working standard solution:

From the standard stock solution(1000 µg/ml) 10 ml of aliquot was transferred into 100ml volumetric flask and volume was filled up to the mark with distilled water to obtain 100µg/ml (2^0 stock) LOS.P and HTZ separately . From the above solution suitable amount of aliquots were withdrawn and mixed them to obtain 8µg/ml of LOS.P and 4µg/ml of HTZ.

Formation of simultaneous equation

For the simultaneous equation LOS.P and HTZ mixed standard solution were prepared which contains both drugs in the concentration ratio of 8:4 µg/ml and it was prepared by appropriate dilution from the individual stock solutions with HPLC- grade distilled water. The absorbances of the mixed standard solutions were measured at their selected wavelengths and pair of simultaneous equation was used for the determination of the concentrations of LOS.P and HTZ from the mixed standard solutions.

$$C_X = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2 \qquad C_y = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2$$

Where C_x and C_y are concentrations of LOS.P and HTZ respectively.

A_1 = absorbance of HTZ at 272 nm. A_2 = absorbance of LOS.P at 215 nm. ax_1 = absorptivity of HTZ at 272 nm ax_2 = absorptivity of HTZ at 215 nm. ay_1 = absorptivity of LOS.P at 272 nm. ay_2 = absorptivity of LOS.P at 215 nm

Analysis of fixed dose tablet

A fixed dose tablet (each tablet contains 50 mg LOS.P and 12.5 mg of HTZ) was analyzed using the developed optimized method. 10 tablets were weighed, finely ground and take the tablet powder equivalent to 10 mg of LOS.P and 2.5 mg of HTZ in a separating funnel and add 10 ml of hot methanol, extract the LOS.P and HTZ for 1hr. Transfer the total content from the separating funnel into a 100ml volumetric flask after filtration dilute the solution with distilled water (1^0 stock). Pipette out 1ml and transfer to 10ml volumetric flask and made up the volume up to the mark with distilled water (2^0 stock). From this 2^0 stock solution pipette out 5ml and transfer to a 10 ml volumetric flask and make up the volume up to 10 ml with distilled water. Measure the absorbance of final solution with at 215nm and 272nm. Apply the simultaneous equation for the calculation of drug content. Results are cited in table 1.

Table.1. Estimation of LOS.P and HTZ in tablet dosage form

Drug	Brand name	Lable claim	Amount of drug estimated	% of label claim estimated
LOS.P	Cosart-H	LOS.P 50mg	49.60mg	99.2 %
HTZ		HTZ 12.5mg	11.32mg	90.6 %

Accuracy:

Accuracy of the present method was performed by recovery study. Here, fixed dose formulation for both analytes was kept constant and standard LOS.P and HTZ were spiked at three different concentrations to calculate 80%, 90%, 120% recovery.

Precision:

It was evaluated by analyzing the six sample solution (n=6) of a 10 mcg LOS.P and HTZ. The inter-day and inter-day precision was determined by measuring six times on same day (inter-day study) and repeated on the next day (intra-day study). The absorbances were recorded and the percentage relative standard deviation (% RSD) calculated for both the drugs separately.

Linearity:

This study was done by preparing the different concentrations (1- 20 µg/ml) for LOS.P and HTZ. The prepared solutions were scanned in the range of 200-400 nm and absorbance measured at 215nm and 272nm against blank. The calibration curve has been plotted considering concentrations Vs absorbance. Obtained data were subjected to regression analysis.

Robustness:

It was performed by preparing six sample solutions of LOS.P and HTZ and analyzed under established conditions like detection wavelength at three different levels for LOS.P and HTZ

Detection and quantitation limit

The standard solution of LOS.P was prepared by sequential dilution and subjected to spectrophotometric analysis using the developed method in the range of 1-20 µg/ml for LOS.P and HTZ. Signal to noise ratio 3.3 was considered for limit of detection (LOD) and 10 for limit of quantification (LOQ).

RESULTS

Method optimization:

Before selecting the conditions for optimization, several preliminary trials were conducted with different solvents (water, methanol, ethanol, acetonitrile, 0.05N HCL, 0.05N NaOH, 0.1N NaOH) in various ratios. Due to less absorptivity, obtaining λ_{max} near solvent UV cut off, irregularities of λ_{max} and spectrum shape, failure of maintaining the linearity most of the solvents has not been considered for optimization. Furthermore some of the solvents (E.g. Ethanol and water) in different ratios lost their justification as they failed as appropriate solvents during validation proceedings. Finally the solvent methanol and water (5 : 95) volume ratio was found optimal, under this condition both analytes maintain linearity and shows justifiable results during validation proceedings, LOS.P and HTZ shows the maximum absorption at 215nm and 272nm respectively (shown in figure 2. Hence these conditions were selected as optimized condition.

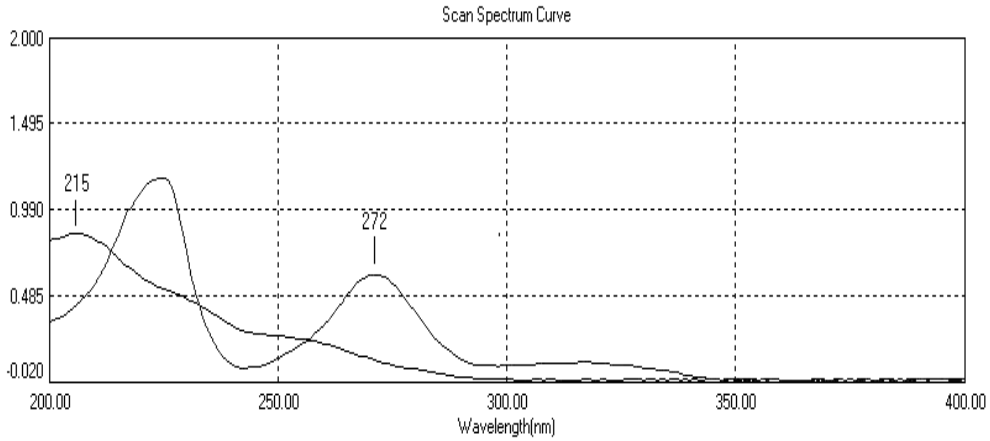

Fig. 2. Overlay spectra of LOS.P and HTZ

Method validation:

The developed method was validated as per ICH guidelines, linear, correlation was absorbed between the concentration and absorbance in specific range. The regression analysis data was present in table 2, the regression coefficient (R^2) was found 0.980 for LOS.P and 0.992 for HTZ. The linearity graphs for both analytes were depicted in figure. The recovery study was performed and accuracy was checked at 80%, 100% and 120%, LOS.P mean recovery study is equal to six (n=6) was 91.55%, 86%, and 90.18% where as HTZ shows 84.91%, 95.38% and 85.47% respectively. In intra-day precision analysis (n=6) the % of RSD value of LOS.P is 0.887 to 1.715, and for HTZ the % of RSD is 0.011 to 0.040 where as the % of RSD for inter-day precision was 1.21 to 1.74 for LOS.P and 0.99 to 1.86 for HTZ, represented in table 3. The developed method shows Robustness on changing the detecting wavelength from 213 to 217 nm for LOS.P and 270 to 274 nm for HTZ respectively. The Robustness study was summarized in table 2, the detection limit was found 0.09 for LOS.P and 0.32 for HTZ. The limit of quantification of the developed method was found 0.248 for LOS.P and 0.97 for HTZ, summarized in table 2.

Table 2: Study of validation Parameters

Sl. No	Parameters		LOS.P	HTZ
1	Accuracy (% recovery)		86-91.55	84.91-95.36
2	Precision (% RSD)	Intraday	0.887-1.715	0.011-0.040
		Interday	1.21-1.74	0.99-1.86
3	Range		1-20	2-20
4	LOD		0.32	0.09
5	LOQ		0.97	0.248
6	Linearity		1-20 µg/ml	2-20 µg/ml
7.	Correlation coefficient (R^2)		0.980	0.992

Table. 3. Results of robustness study

	*Mean Absorbance LOS.P		*Mean Absorbance HTZ		*Mean Absorbance LOS.P	*Mean Absorbance HTZ
parameters	λ_{max} 213nm	λ_{max} 217nm	λ_{max} 270nm	λ_{max} 274nm	Solvent ratio (Water: Methanol) 97:3	Solvent ratio (Water: Methanol) 93:7
Mean	0.578	0.686	0.341	0.336	0.495	0.289
SD	0.008	0.001	0.001	0.008	0.002	0.016
% RSD	0.138	0.145	0.293	0.2381	0.223	0.486

**average of six replicates*

DISCUSSION

In this present proposed method has been well optimized under the experimental conditions and solvent methanol: water (5:95) and detection wavelength 215 for LOS.P and 272 for HTZ exhibits sufficient absorbance reliable sensitivity, linearity and excellent spectrum shape. The optimized conditions were allotted for subsequent study of validation parameters. The developed method was found accurate in all three levels (80%, 100%, 120%), the results

of both intra-day and inter-day precision study was found very precise as % of RSD values were less than 2. The obtained regression coefficient values 0.980 for LOS.P and HTZ, indicates the linearity of the method. The results of Robustness study shown that small changes in operation parameter do not affect the results significantly. The LOD and LOQ studies demonstrated the sensitivity of the proposed method. The assay results of the marketed tablets indicates the efficiency of the proposed simultaneous equation method for determination of drug content from the pharmaceutical dosage form.

CONCLUSION

The present method was found very simple because the use of methanol and water as a solvent and it is also economic and easily available. The use of large volume of distilled water as an analyzing solvent makes the method cheap. The method has been successfully validated as all validation parameters as per ICH guidelines were found within acceptable limitations. So, this method is reliable for the simultaneous drug content of LOS.P and HTZ in bulk and tablet dosage form in quality control laboratory for routine analysis.

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