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Original Research Article

Development and Validation of Analytical Method for Losartan-Copper Complex Using UV-Vis Spectrophotometry

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Abstract

Purpose: *To develop a new spectrophotometric method for the analysis of losartan potassium in pharmaceutical formulations by making its complex with copper.*
Method: *A coloured complex based on UV/Vis spectroscopic method was developed for the determination of losartan potassium concentration in pharmaceutical formulations in the visible region. The colored complex of losartan was formed with cupric acetate (5:4). Analysis was carried out by the two methods - absorption ratio and calibration curve methods. The proposed method was validated in terms of linearity, accuracy and precision.*
Results: *The λ maximum of the complex was recorded at 530 nm. Beer`s law was obeyed in the range of 10 - 50 µg/ml with a correlation coefficient (r^2) of 0.9989. Mean analytical results from the calibration curve and absorption ratio methods were 99.42 and 99.16 % with relative standard deviation (RSD) of 0.97 and 0.82 %, respectively. Mean recovery was between 98.61 and 101.35 % and precision expressed as relative standard deviation (RSD) was 0.91 %.*
Conclusion: *The proposed method is simple, easy to apply, low-cost, and requires relatively inexpensive instruments. Thus, it is a suitable alternative to currently used spectrophotometric methods for the determination of losartan in bulk and solid dosage forms.*

Keywords: *Losartan, Copper complex, Spectrophotometry, Validation*

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INTRODUCTION

The potassium salt of losartan, 2-n-butyl-4-chloro-5-hydroxymethyl- 1-((2-(1H-tetrazol-5-yl) (biphenyl-4-yl) methyl) imidazole, is a strong antihypertensive angiotensin II receptors blocker [1-3]. Copper is an essential transition element that plays a major role in the biochemistry of all aerobic organisms. This metal triggers a series of electron transfer reactions as a cofactor in a number of critical enzymatic pathways. These enzymes are essential for cellular respiration, iron homeostasis, pigment formation, neurotransmitter production, peptide biogenesis, connective tissue biosynthesis, and antioxidant defense [4,5].

Molecular interactions between electron donors and electron acceptors are generally associated with the formation of intensely colored charge-transfer complexes, which absorb radiation in the visible region [6]. The rapid formation of these complexes leads to their utility in the development of simple and convenient spectrophotometric methods for these

compounds [7-9]. The copper (II) complex of Losartan was obtained and characterized as a microcrystalline powder. Vibrational spectroscopy reveals that the metal center is bound to the ligand through the nitrogen atoms of the tetrazolate moiety. The antioxidant properties of the complex (superoxide dismutase mimetic activity) and its effect on the proliferation and morphology of two osteoblast-like cells in culture have been reported. The new compound exerted more toxic effects on tumoral cells than the copper (II) ion and losartan [10].

Analysis of losartan has been carried out in tablets and biological fluid by high performance liquid chromatography (HPLC) [11,12], capillary electrophoresis, super-critical fluid chromatography [13,14], and in urine by gas chromatography-mass spectrometry [15]. However, to the best of our knowledge, no UV-Vis spectrophotometric method has been reported for the analysis of losartan-copper. The aim of the study, therefore, was to develop an easy, economical and sensitive spectrophotmetric method for the determination of losartan potassium in pharmaceutical preparations by complex it with copper.

EXPERIMENTAL

Materials

Losartan potassium was received from Mass Pharma (Pvt) Ltd, Lahore, Pakistan as gift. Cupric acetate and glacial acetic acid (both manufactured by E. Merck Germany) were purchased from a local source.

Complex formation

The method was constructed on the basis of drug complex analysis by UV/VIS spectrophotometer. For the preparation of drug complex, pure losartan potassium and cupric acetate were chosen. Different ratios of losartan potassium and cupric acetate for complex formation were prepared as indicated in Table 1. The complex was formed by first mixing varying amounts of accurately weighed losartan potassium with 50 ml of distilled water and filtered through Whatman filter paper (0.45 µm). Cupric acetate (varying amounts) was added to the filtrate with slight shaking for 5 min to achieve formation of the blue-colored complex of losartan potassium (Table 1). The colored complex was filtered through Whatman filter paper, ,dried at room temperature and accurately weighed. The drug/cupric acetate ratio that yielded the highest complex weight was chosen for method development.

Table 1: Preparation of losartan – copper complex.

Losartan potassium (mg)	Cupric acetate (mg)	Weight of complex formed (mg)
100	50	2.12
100	60	2.57
100	80	3.58
100	100	3.56

Selection of an appropriate solvent system

Various solvent systems, including distilled water, Ethyl alcohol, Acetone, Cholorom and Glacial acetic acid, were tested to select an appropriate solvent with good solubility and stability of drug–copper complex. Glacial acetic acid was selected for developing spectral characteristics of drug due to the solubility of losartan-copper complex in this liquid.

Determination of absorption maximum

The color of copper is brownish showing absorption maximum (*λ*max) at 740 nm. Upon formation of complex, this color was immediately turned bluish and the absorption spectrum of losartan potassium-copper reaction product showed maximum absorption peak at 530 nm (Figure 1). This change in color indicated the formation of a charge-transfer complex between losartan potassium and copper.

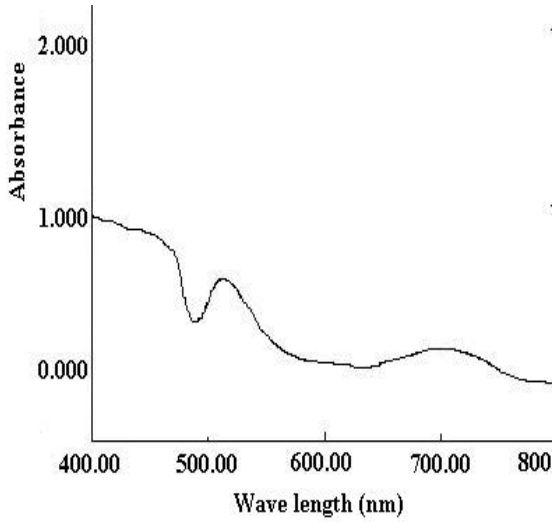

Figure 1: Spectrum of losartan–copper complex showing absorption maximum at 530 nm

Preparation of standard solution

Pure losartan potassium powder (100 mg) was dissolved in 25 ml of water in a beaker and the solution filtered through Whatman filter paper; 80 mg of cupric acetate was added to the filtrate and shaken for 5 min, producing a light blue complex. The complex was filtered through Whatman filter

paper, dried at room temperature and weighed. The whole complex formed was dissolved in a small amount of glacial acetic acid (10 ml), transferred to a 100 ml volumetric flask, made up to 100 ml with glacial acetic acid and shaken well. From this solution 10 ml was transferred to another 100 ml volumetric flask and made up to 100 ml with glacial acetic acid. Finally, 10 ml of this solution was transferred to yet another 100 ml volumetric flask and the volume again made up to 100 ml with glacial acetic acid.

Preparation of sample solution

Twenty tablets of losartan potassium of 50 mg strength each were weighed and ground them using a pestle and mortar. A portion of the powder containing 100 mg of losartan potassium was added to 25 ml of water, shaked well. Solution was then filtered. 80 mg of cupric acetate was added to the filtrate and shaken for 5 min. A light blue color complex was formed which was then filtered, dried, dissolved in a small amount of glacial acetic acid (10 ml) and transferred to a 100 ml volumetric flask. The volume of the solution was made up to the 100 ml mark with glacial acetic acid. Out of this solution, 10 ml was transferred to another 100 ml volumetric flask and made up to 100 ml with glacial acetic acid. Finally, 10 ml of this was transferred to another 100 ml volumetric flask and made up to 100 ml with glacial acetic acid.

Calibration curve method

A calibration curve for losartan potassium copper complex, which obeyed Beer's law was plotted. Absorbance was determined spectrophotop-metrically using UV-2550, Shimadzu, Japan spectrophotometer at 530 nm and the drug content of the samples were computed

Absorption ratio method

The 530 nm wavelength was selected from the scanned spectrum of losartan copper complex, being the λ max of drug-copper complex .The ratio of the absorbance of the sample solution to that of the standard solution, expressed as a percentage, was taken as purity (%)

Validation of the method

The method was validated according to the ICH guidance in terms of linearity, accuracy, precision.

Linearity

Linearity of the proposed methods was determined by analyzing the samples of losartan copper complex in the range of 10 - 50 µg/ml.

Accuracy

Accuracy of analysis was determined by systemic error method based on the closeness between the actual (true) value and analytical value and was obtained by applying the test in six replicates

Accuracy may often be expressed as % recovery. This method is the measure of the exactness of the analytical method. Recovery experiment was carried out in triplicate by analyzing samples of the losartan copper complex (10 µg/ml) at three different concentrations of standards (1, 2, 3 µg/ml).

Precision

System precision was estimated from repeatability and reproducibility. The Intraday and interday precision of the method were confirmed by measuring absorbance of six replicate samples of losartan copper complex three times in a day and also on two different days. Percentage relative standard deviation (% RSD) values should be < 2 %.

Statistical analysis

Data analysis was carried out using ANOVA using software, SPSS version 13.0. The level of significance was set at $p < 0.05$.

RESULTS

Mean drug content

The assay results from calibration curve and absorption methods showed 99.42 and 99.16 % losartan content, respectively; the difference was non-significant ($p > 0.05$) and was not also different from the stated purity for the standard drug. These results indicate good accuracy of method (Table 2).

Table 2: Results of analysis of tablet samples

Analyte	Calibration curve method	Absorption ratio method
Conc.	99.42 %	99.16 %
RSD	0.97	0.82

Linearity

The calibration curve had a small intercept (-0.01) and was linear in the concentration range

of 10 – 50 µg/ml with a correlation coefficient of 0.9989.

Accuracy

Percent recovery for losartan was found to be in the range of 98.61 to 101.35 % (Table 3).

Table 3: Recovery data for losartan potassium (mean ± SD).

Analyte	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery (mean±SD)
Losartan copper complex	10	1	10.08	101.35±1.22
	10	2	11.98	98.84±0.87
	10	3	12.82	98.61±1.72

Precision

Precision was studied to find out intra- and inter-day variation. Calibration curves prepared in the medium were run three times on the same day and continued for three days. %RSD (relative standard deviation) were calculated. Precision was < 2 %, and the data are given in Table 4.

Table 4: Results of intermediate precision (mean ± %S.D)

Variation	Calibration curve Method	Absorption ratio Method
	% Label claim	*% Label claim*
Intra-day	101.40±0.75	99.89±0.66
Inter-day	100.98±1.0	100.82±1.26

DISCUSSION

Various researchers have studied losartan potassium separately or in combination with other drugs in various dosage forms [14,16,17]. However, to the best of our knowledge, no analytical study regarding the determination of losartan potassium as a complex with copper ions using spectrophtometric technique has been reported. The complexes of drugs always showed better biological effects than drug alone. Based on previous studies, Saeed *et al* [18] synthesized the drug-enzyme conjugate complex of dexamethasone – subtilisin enzyme and dexamethasone- cellulase in order to study their drug - protein ratio, immunoreactivity, enzyme activity, stability and proved that the drug - enzyme complex can be synthesized and characterized for better activity. Saeed *et al* [19] further investigated the enzyme activities for pepsin and its complexation with dexamethasone to study the immunoreactiv by rapid enzyme linked immunosorbent (ELISA) assay which proved that the complex was immunoreactive to dexamethasone specific antibodies. This study was useful for preparing a variety of drug - complex moieties.

Losartan does not show antioxidant activity, its copper complex has displayed a moderately anti tumour activity. This effect cannot produced by the copper ion or the Losartan ligand itself even in higher concentration. So the resultant complex produces more toxic action on the tumour cells than the copper ion and losartan [10].
Ibrahim [20] reported an analytical study for the charge-transfer complexes of losartan potassium. The method was successfully applied to the analysis of tablets from different manufacturers that contain LOS-K, alone or combined with hydrochlorothiazide, with good accuracy and precision; recovery values were in the range of 98.96 ± 1.62 to 101.58 ± 1.29 %.
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The aim of the present study was to carry out charge-transfer complexation reaction of losartan potassium (LOS-K) as electron donor and copper which is an electron acceptor. The accuracy of the method was confirmed by recovery results for tablet at three different levels of standard additions, in range of 95 – 110 % justifies the accuracy of this method. Olga *et al* [16] presented an analytical UV derivative spectrophotometric method to quantify losartan potassium used as a single active principle in pharmaceutical dosage forms. The mean recovery of the method was 100.7 ± 1.1 % and the precision (expressed as relative standard deviation) was 0.88 %.

The present method is superior to previously reported UV-based spectrophotometric methods, because the measurements were performed in the visible region, away from the UV-absorbing region. Consequently, the colored complexes obtained can be utilized in the determination of of LOS-K in different pharmaceutical tablet dosage form.
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CONCLUSION

The charge-transfer complexation reaction of losartan potassium with copper has successfully been utilized in the development of a simple, rapid and accurate spectrophotometric method for the analysis of losartan potassium in pharmaceutical dosage forms. The developed methods, unlike previously reported methods, are not UV-based spectrophotometric methods, as the measurements were performed in the visible region. The developed method would be useful for routine quality control analysis of losartan potassium tablets.

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