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VALIDATED HPTLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LOSARTAN POTASSIUM AND CHLORTHALIDONE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

This present study reports for the first time simultaneous quantification of Losartan Potassium and Chlorthalidone in tablet dosage form by employing High Performance Thin Layer Chromatographic method. Chromatographic separation of the drugs were performed on aluminium plates precoated with silica gel 60 G F254 as the stationary phase and the mobile phase used was a mixture of Chloroform: Methanol: Ammonia (9: 2: 0.2, v/v). Densitometric evaluation of the separated zones was performed using a UV detector at 254 nm in absorbance mode. The Rf values for Losartan Potassium and Chlorthalidone were found to be 0.43±0.01 and 0.82± 0.01 respectively. The calibration curve was found to be linear between 1.4 - 2µg/spot for Losartan Potassium and 0.5 – 1.1µg/spot for Chlorthalidone. The method was specific because no chromatographic interferences from the tablet excipients were found. The accuracy and reliability of the method was assessed by recovery studies, precision (intraday and interday) and linearity in accordance with ICH guidelines. The limits of detection and quantification were found to be 23.17ng/spot and 70.21ng/spot for Losartan Potassium, and 4.92ng/spot and 14.92ng/spot for Chlorthalidone, respectively. The developed method can be successfully employed for the simultaneous determination of these drugs in tablet formulation.

KEY WORDS

Losartan Potassium, Chlorthalidone, Method development, High performance thin layer chromatography

INTRODUCTION [1, 2]

Losartan Potassium (LOS), chemically 2-Butyl-4-chloro-1-[(2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl)methyl]-1H-imidazole-5-methanol mono potassium salt(Fig.1) is the first orally active Angiotensin II receptor antagonist available for the treatment of hypertension and Chlortalidone (CTD), 2-Chloro-5'-(1-hydroxy-3-oxo-1-isoindolinyl)benzene sulfonamide(fig.2) is long acting thiazide like antihypertensive diuretic used in the treatment of edema associated with congestive heart failure.

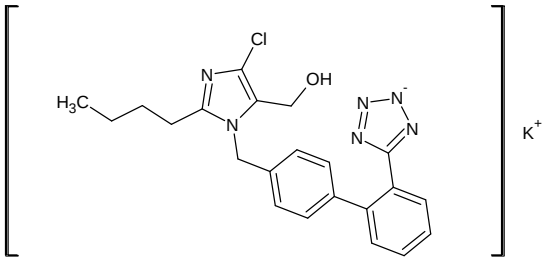

Fig.1.Structure of Losartan Potassium (LOS)

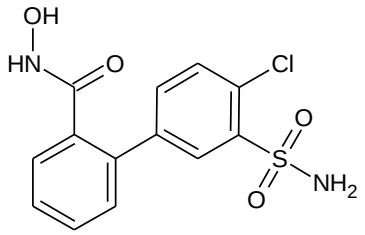

Fig.2.Structure of Chlortalidone (CTD)

Compared with monotherapy, combination therapy has the advantages to be more effective and more prompt in BP lowering, with fewer adverse events. Lower minimal doses of diuretics are effective. Lower maximal doses of diuretics reduce complications. The Losartan/low-dose Chlorthalidone combination is effective in lowering BP and is well tolerated, thus providing a useful therapeutic option for treating mild-to-moderate hypertension.

Literature review revealed that very few methods are reported for the simultaneous estimation of Losartan Potassium and Chlorthalidone which include high-performance liquid chromatography, ratio derivative method and area under curve method. The goal of present work is to develop HPTLC method that could be applied in quality control laboratories for the simultaneous determination of both drugs.

MATERIALS AND METHODS

Materials

Reference standards of Losartan Potassium and Chlortalidone

HPLC grade solvents: Methanol (S D fine-chemicals limited, Mumbai), Chloroform (Ranbaxy fine-chemicals limited, New Delhi), Ammonia (Himedia Laboratories Pvt.Ltd. Mumbai). Marketed combination product of Losartan Potassium and Chlorthalidone (Covance-CT containing 50mg Losartan Potassium and 12.5mg Chlortalidone marketed by Ranbaxy Laboratories Pvt. Ltd.)

Instrument and Software

CAMAG HPTLC system comprising of Linomat IV automatic sample applicator, CAMAG Hamilton microlitre syringe (100 µl), CAMAG TLC scanner III with WINCATS software, Stationary phase: HPTLC pre coated plates; silica gel G60 F_{254} (20x20), Shimadzu analytical balance and Ultrasonicator were used.

Methods

Preparation of standard solutions

Standard solutions of Losartan Potassium RS in methanol

Weighed accurately 50mg of Losartan Potassium RS and transferred to a 50ml standard flask. It was dissolved in HPLC grade methanol and made upto the volume. This solution had a concentration of 1000µg/ml.

From the above solution 7ml, 8ml and 9ml were pipetted out into three numbered 10ml standard flask and volume was made upto the mark with methanol to get a concentration of 700 µg/ml, 800 µg/ml and 900 µg/ml.

Standard solutions of Chlorthalidone RS in methanol

Weighed accurately 50mg of Chlorthalidone RS and transferred to a 50ml standard flask. It was dissolved in HPLC grade methanol and made upto the volume. This solution had a concentration of 1000µg/ml.

From the above solution 2.5ml, 3.5ml, 4.5ml and 5.5ml were pipetted out into four numbered 10ml standard flask and volume was made upto the mark with methanol to get a concentration of 250 µg/ml, 350 µg/ml 450µg/ml and 550µg/ml.

Preparation of standard drug mixture:

50 mg of Losartan Potassium RS and 12.5 mg of Chlorthalidone RS were weighed separately and transferred into a 50ml standard flask. The drug mixture was allowed to dissolve in sufficient quantity of methanol by shaking for 15 min and the volume was made upto the mark with methanol to obtain a mixture with concentration of 1000µg/ml of Losartan Potassium and 250µg/ml of Chlorthalidone.

Development of solvent system

The mobile phase was selected based on the polarity of analytes (Losartan Potassium and Chlorthalidone) and absorption property of silica gel plates. The suitable solvent system was selected by a series of trial and error process. By the trial and error process, a solvent system of Chloroform: Methanol: Ammonia in the ratio 9:2:0.2 was selected for the HPTLC analysis of Losartan Potassium and Chlorthalidone in the combined form.

Preparation of sample solution

Twenty tablets of Covance-CT were weighed; average weight of one tablet was determined and finely powdered with the help of mortar and pestle. A quantity of powder equivalent to 50mg of Losartan Potassium, and 12.5mg of Chlorthalidone was accurately weighed, transferred to a stoppered flask and extracted with 20ml of methanol initially by shaking vigorously for 15 minutes. The solution was transferred to a 50 ml standard flask through a Whatman No. 1 filter paper. The residue was then further extracted twice with 10 ml methanol and transferred to the same standard flask through the same filter paper. The volume was finally made upto 50 ml with methanol. The resulting solution had a concentration of 1000µg/ml of Losartan Potassium, and 250µg/ml of Chlorthalidone as per label claim.

Selection of Wavelength for Measurement

After chromatographic development, the spots were scanned over the range of 200 - 400 nm and the spectra were overlain. It was observed that, the drugs showed considerable absorbance at 254 nm. So, 254 nm was selected as the wavelength for detection.

Chromatographic Condition

Stationary Phase	:	Precoated silica gel G60 F254 aluminium sheet (20 × 10 cm)
Mobile Phase	:	Chloroform: Methanol: Ammonia, 9:2:0.2
Chamber Saturation Time	:	30 min.
Temperature	:	Ambient Temperature
Start position	:	12mm
Bandwidth	:	4 mm
Space	:	16mm
Application volume	:	2µl
Flow rate	:	2µl/sec.
Detection	:	Densitometrically using a UV detector at 254 nm.

RESULTS AND DISCUSSIONS

A simple, accurate and precise HPTLC method was developed and validated for the simultaneous estimation of Losartan Potassium and Chlorthalidone. The proposed HPTLC method was optimized with several solvent systems. The mobile phase consisting of Chloroform: Methanol: Ammonia (9: 2: 0.3, v/v) gave sharp and symmetrical peaks with the Rf values 0.43, and 0.82 for LOS and CTD

respectively. HPTLC chromatograms of individual drugs (fig.1 and fig.2) and resolution of the peaks for mixture of standard drugs and sample solution with clear baseline separation was obtained (Fig.3 And Fig.4). A 3-D chromatogram showing peaks of LOS and CTD in different concentrations at 254 nm is depicted in Fig.5. The calibration curves for LOS and CTD were constructed by plotting peak area and concentration and the amount of LOS and CTD in the drug mixture and sample solutions were determined from the computed regression equations.

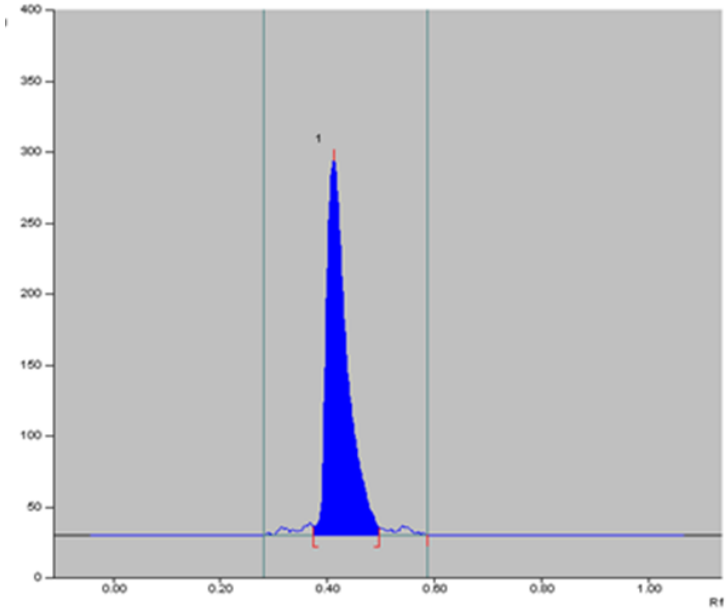

Fig.3 Chromatogram of Losartan Potassium

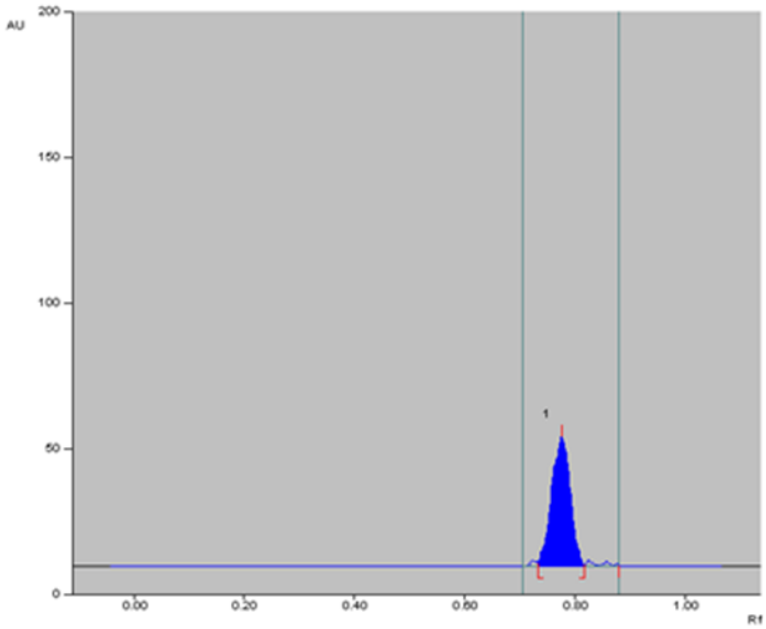

Fig.4 Chromatogram of Chlorthalidone

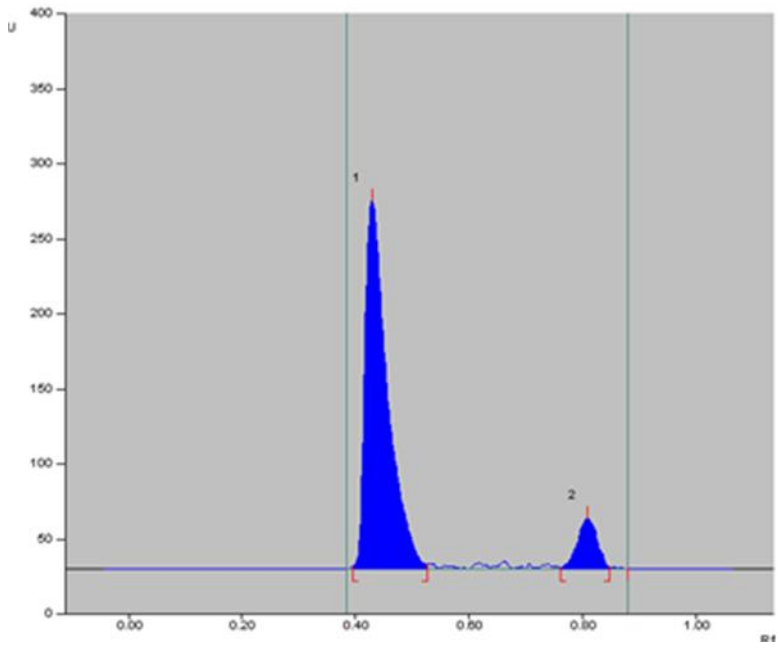

Fig.5 Chromatogram of standard drug mixture

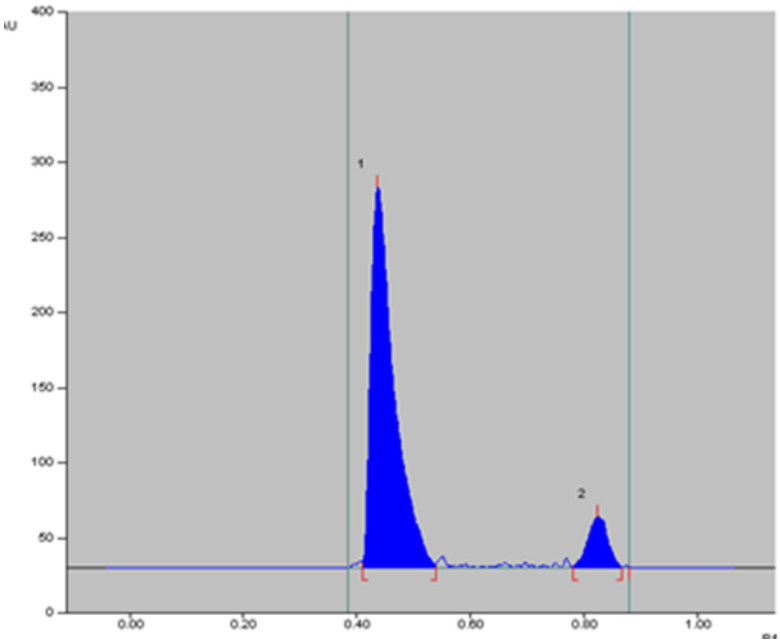

Fig.6 Chromatogram of sample drug

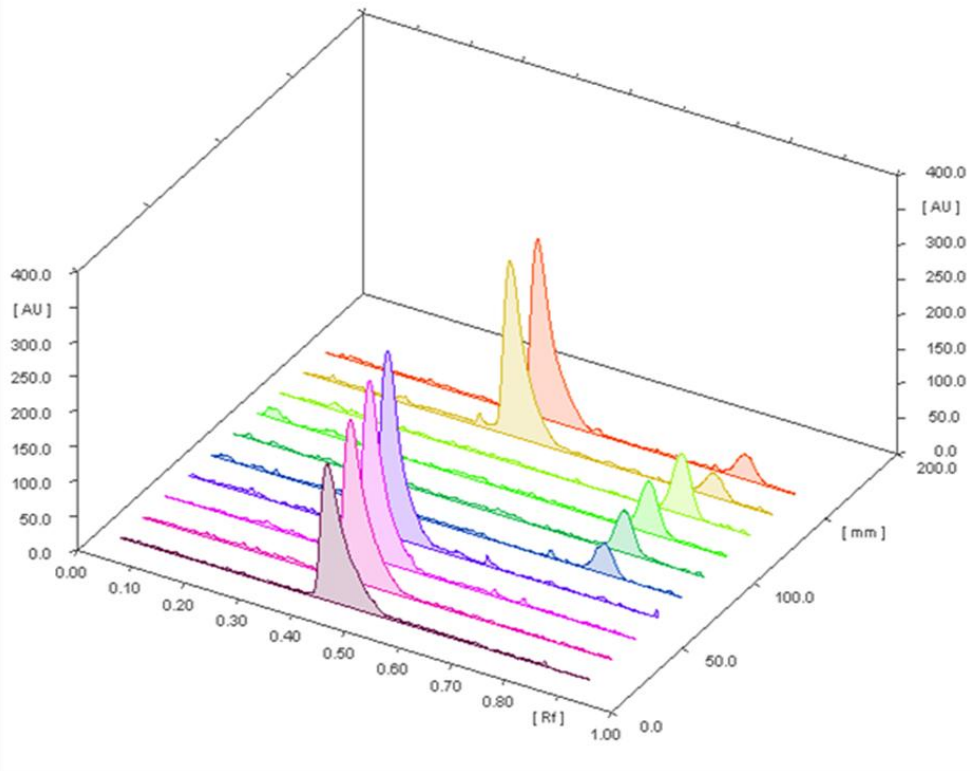

Fig.7 3D overlay spectra

Method validation

The method was validated according to ICH Q2 (R1) guidelines. The following parameters were used for validation of the proposed method. Summary of validation parameters were tabulated in Table 1.

Linearity

Linearity was checked by preparing standard solutions of LOS and CTD at four different concentration levels. The calibration curves were drawn in the concentration range of 1400-2000ng/spot for LOS and 500-1100ng/spot for CTD. The calibration curves were constructed by plotting peak area versus concentration (Fig.6 and Fig.7). Each reading was the average of three determinations. The correlation coefficient (R^2) for calibration curve LOS and CTD were found to be 0.996 and 0.999 respectively.

Accuracy

The accuracy of the method was determined by calculating recoveries of LOS and CTD by the standard addition method. For that, known amounts of standard solutions at 80, 100 and 120 % level were added and analyzed by the proposed method, in triplicate.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by using 100%test concentration. Chromatograms were developed using mixed standard solution containing 1000µg/ml LOS and 250µg/ml CTD. The peak area was scanned six times at 254nm on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

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

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the six replicate determinations, y intercept

was calculated and the standard deviation of the y intercept was computed. From these values, Limit of detection (LOD) and Limit of quantitation (LOQ) were determined on the basis of response and slope of the regression equation. LOD and LOQ were calculated using following equation as per ICH guidelines. LOD = 3.3 × σ/S, LOQ = 10 × σ/S, where σ is the standard deviation of y intercepts of regression lines and S is the slope of calibration curves.

Robustness

In order to establish the robustness of the method, small deliberate changes were made in the experimental conditions and chromatographic parameters like mobile phase composition (±0.1 ml for each component), the plate activation time, chamber saturation time (±10% change from set time), volume of mobile phase (±10% change from set volume) and the development distance (±10% change from set distance). The time from spotting to development (0, 10, 20, 30 min) and from development to scanning (0, 10, 20, 30 min) was also varied. In the above changed conditions, stock solution was analyzed and results of robustness studies were expressed in term of % RSD of peak areas in each changed condition and were compared with similar results obtained in unchanged experimental conditions.

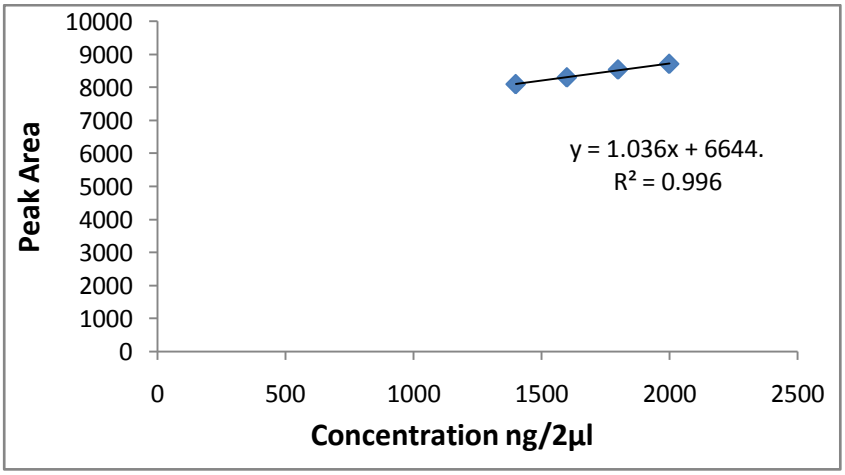

Fig.8 Calibration graph of Losartan Potassium: concentration v/s Peak area

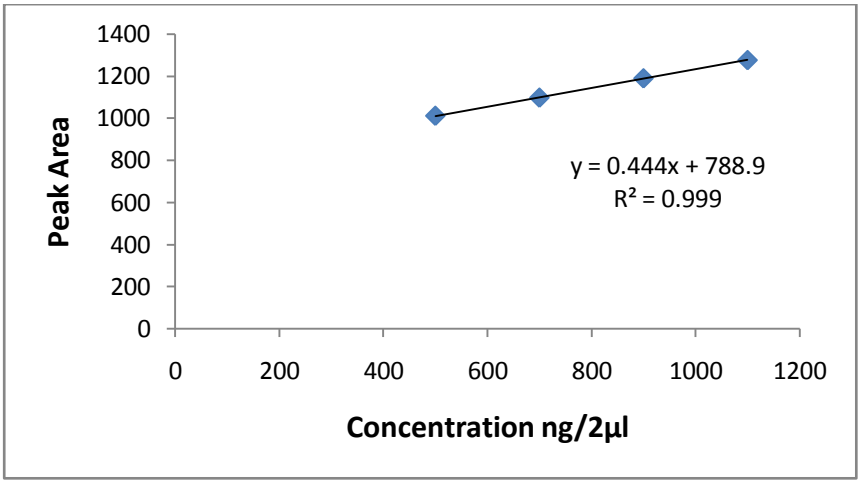

Fig.9 Calibration graph of Chlorthalidone: Concentration v/s Peak area

Table1. Summary of validation parameters

Parameters		HPTLC method	
		LOS	CTD
Linearity (ng/spot)		1400-2000	500-1100
Slope		1.03	0.444
Intercept		6644	788.9
Correlation coefficient		0.996	0.999
Accuracy (%recovery)	80%level	0.1847	0.6155
	100%level	0.0291	0.1033
	120%level	0.0805	0.0385
Repeatability	%RSD intraday	0.0032	0.0080
	%RSD interday	0.0002	0.0003
LOD (ng/spot)		23.17	4.92
LOQ (ng/spot)		70.21	14.92

Assay of marketed formulation

The proposed method was applied for the estimation of LOS and CTD in marketed formulations and assay results shown in Table 2.

Table 2: Assay result of marketed formulation

Formulation	Label claim(mg/tablet)		Amount found (mg)		Amount found (%)	
Covance-CT	LOS	CTD	LOS	CTD	LOS	CTD
	50	12.5	49.44	12.43	98.88	99.44

CONCLUSION

The results of analysis of pharmaceutical dosage form by the proposed method are highly reproducible, reliable and are in good agreement with label claim of the drug. The proposed method does not suffer from any interference due to common excipients. It indicates that method is accurate. Therefore the proposed method could be successfully applied to estimate commercial pharmaceutical products containing LOS and CTD.

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