

## DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF TELMISARTAN AND CHLORTHALIDONE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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### ABSTRACT

A simple, accurate and precise densitometric method for the simultaneous estimation of Telmisartan and Chlorthalidone in Bulk and Pharmaceutical Dosage form has been developed and validated. Separation of drugs was carried out using Acetonitrile: Toluene: Glacial acetic acid (7.5: 2.5: 0.05 v/v/v) as mobile phase on precoated Silica Gel 60F254 plates. The densitometric evaluation of spots was carried out at 242 nm. The  $R_f$  value for Telmisartan and Chlorthalidone were found to be  $0.26 \pm 0.02$  and  $0.67 \pm 0.02$ , respectively. The method was validated with respect to linearity, accuracy, precision and robustness as per the International Conference on Harmonisation (ICH) guidelines. The drug response with respect to peak area was linear over the concentration range 400-2400 ng/spot (n=6) and 125-750 ng/spot (n=6) for Telmisartan and Chlorthalidone respectively. The limit of detection and limit of quantitation were found to be 9.05 ng/spot and 27.42 ng/spot for Telmisartan and 5.15 ng/spot and 15.6 ng/spot for Chlorthalidone. The percentage recovery of Telmisartan and Chlorthalidone was found to be 100.03 and 100.026 respectively. The % R.S.D. values for intra-day precision study and inter-day precision study were <1.0%, confirming that the method was sufficiently precise. The method can be successfully employed for the simultaneous determination of Telmisartan and Chlorthalidone in pharmaceutical formulations.

**Keywords:** Telmisartan, Chlorthalidone, HPTLC, Simultaneous determination, Validation.

### INTRODUCTION

Telmisartan (TEL) is chemically 4'-[[4-methyl-6-(1-methyl-2-benzimidazolyl)-2-propyl-1-benzimidazolyl]methyl]-2-biphenylcarboxylic acid (Fig. 1) is a Antihypertensive drug [1-7]. It is an Angiotensin II receptor antagonist. It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and U.S. Pharmacopoeia (USP). It is estimated by Liquid Chromatography as per IP and Potentiometric titration as per BP and USP [5-7]. Literature review reveals that HPLC [9-13], UV [14-16] spectrophotometric and HPTLC [17-23] methods has been reported for estimation of TEL in pharmaceutical dosage forms. Chlorthalidone (CHL) is chemically (RS)-2-chloro-5-(1-hydroxy-3oxoisindolin-1-yl)benzenesulphonamide (Fig.2). It is a diuretic drug used to treat hypertension [1-7]. It is official in IP, BP and USP and estimated by potentiometric titration as per IP and Liquid Chromatography as per BP and USP [5-7]. Literature review also reveals that HPLC [24-27], UV [28] spectrophotometric methods has been reported for the estimation of CHL in pharmaceutical dosage forms. Literature survey does not reveal any HPTLC method for simultaneous determination of TEL and CHL in Pharmaceutical dosage form. The present developed HPTLC method is simple, precise and accurate for simultaneous determination of both drugs in their Pharmaceutical Dosage forms as per International Conference on Harmonization (ICH) guidelines [8].

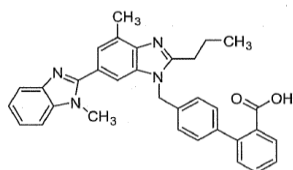


Fig. 1: Structure of Telmisartan (TEL)

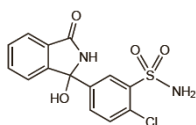


Fig. 2: Structure of Chlorthalidone (CHL)

### MATERIALS AND METHODS

#### Chemicals and Reagents

Pure drug samples of Telmisartan & Chlorthalidone were provided as a gift sample by Alembic Limited, Vadodara, Gujarat, India. Commercial pharmaceutical tablets **ERITEL-CH40** (Eris Lifesciences Pvt. Ltd, Ahmedabad, Gujarat, India) was procured from local pharmacy. Acetonitrile, Toluene, Methanol and Glacial acetic acid of AR Grade. Acetonitrile was obtained from S D Fine Chem Limited, Mumbai, Maharashtra, India and all other chemicals were obtained from Allied Chemical Corporation, Vadodara, Gujarat, India.

#### Instrumentation and Chromatographic conditions

The HPTLC system (Camag, Muttenz, Switzerland) consisted of Linomat V autosprayer connected to a nitrogen cylinder, a twin trough chamber (10 × 10 cm), a derivatization chamber, and a plate heater. Pre-coated silica gel 60 F254 TLC plates (10 × 10 cm, layer thickness 0.2 mm (E. Merck KGaA, Darmstadt, Germany) was used as stationary phase. TLC plates were pre-washed with methanol and activated at 80°C for 5 min prior to sample application. The standard and formulation samples of TEL and CHL in mixture were spotted on Pre-coated TLC plates in the form of narrow bands of lengths 6 mm. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nL/s. The mobile phase consists of Acetonitrile: Toluene: Glacial acetic acid (7.5:2.5:0.05 v/v/v). Linear ascending development was carried out in twin trough chamber (10 × 10 cm). The optimized chamber saturation time for mobile phase was 15 min, at ambient temperature; the length of chromatogram run was 7 cm. Densitometric scanning was performed on CAMAG TLC scanner 3 in Absorbance/Reflectance mode, operated by winCATS 1.3.4 planar chromatography software. The spots were analyzed at a wavelength of 242 nm. The slit dimensions used in the analysis were length and width of 5 mm and 0.45 mm, respectively, with a scanning rate of 20 mm/s. The parameters were selected as recommended by the CAMAG TLC scanner 3 manual. Evaluation was performed using linear regression analysis of peak areas.

#### Preparation of stock, working standard solutions and calibration curves

Accurately weighed Telmisartan (10 mg) was transferred to 10 ml volumetric flask, dissolved in and diluted with methanol up to the

mark (1000 µg/ml). For preparation of CHL stock solution, accurately weighed Chlorthalidone (10 mg) was transferred to 10 ml volumetric flask, dissolved in and diluted with methanol up to the mark (1000 µg/ml). For preparation of working standard solution, 4 ml of stock solution of TEL (1000 µg/ml) and 1.25 ml of stock solution of CHL (1000 µg/ml) were transferred to 10 ml volumetric flask and diluted with methanol upto the mark to obtain final concentration containing 400 µg/ml of TEL and 125 µg/ml of CHL. Calibration was done by applying mixture of standard solutions ranging from 1.0 – 6.0 µl by Hamilton syringe with the help of Linomat V autosprayer on TLC plate that gave concentration 400-2400 ng/spot for TEL and 125-750 ng/spot for CHL, respectively. Each concentration was spotted six times on TLC plates. From the developed plates calibration curve was plotted as peak areas versus corresponding concentrations (Fig. 5 and 6).

#### Analysis of TEL and CHL in marketed Tablet Formulation

To determine the content of TEL and CHL simultaneously in conventional tablets (Eritel- CH40 label claim 40 mg TEL and 12.5 mg CHL); twenty tablets were accurately weighed, average weight determined and ground to fine powder. A quantity of powder equivalent to 40 mg (TEL) and 12.5 mg (CHL) was transferred into 100 ml volumetric flask containing 50 ml methanol, sonicated for 30 min and diluted to mark with same solvent to obtain 0.4 mg/ml of TEL and 0.125 mg/ml of CHL. The resulting solution was filtered using 0.45 µm filter (Millifilter, MA). So, Resultant solution was found to contain 400 µg/ml (400ng/µl) Telmisartan and 125µg/ml (125 ng/µl) Chlorthalidone. 3 µl of this solution was applied on TLC plate followed by development and scanning at 242 nm. The analysis was repeated for three times.

#### Development and Validation of HPTLC Method

##### Linearity

For the linearity study the 1-6 µl from the working standard solution containing 400 ng/spot of TEL and 125 ng/spot of CHL was injected. So, linearity responses for TEL and CHL were assessed in the concentration range 400-2400 ng/spot and 125-750 ng/spot, of working standard solutions, respectively.

##### Precision

Precision of the method was determined in the terms of intra-day and inter-day variation (%RSD). Intra-day precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of 7 days.

##### Accuracy

To the pre-analyzed sample a known amount of standard solution of pure drug (TEL and CHL) was spiked at three different levels (80%,

100% and 120%). These solutions were subjected to re-analysis by the proposed method.

##### Sensitivity

The sensitivity of measurement of TEL and CHL by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by equation. Based on the standard deviation of the response and the slope, LOD and LOQ were estimated using the formulae:

$$LOD = 3.3 \sigma / S$$

Where,  $\sigma$  = the standard deviation of the response

S = the slope of the calibration curve

$$LOQ = 10 \sigma / S$$

Where,  $\sigma$  = the standard deviation of the response

S = the slope of the calibration curve

LOD and LOQ were determined from the standard deviations of the responses for six replicate determinations.

##### Specificity

Specificity of the method was ascertained by analyzing standard drug and sample. The mobile phase resolved both the drugs very efficiently as shown in Fig. 7. The spot for TEL and CHL was confirmed by comparing the  $R_f$  and spectra of the spot with that of standard. The wavelength 242 nm for detecting peak purity of TEL and CHL was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

##### Repeatability

Repeatability of sample application was assessed by spotting 4µL (1600 ng/spot of TEL and 500 ng/spot of CHL) of drug solution six times on a TLC, followed by development of plate and recording the peak area for six spots.

#### RESULTS AND DISCUSSION

The TLC procedure was optimized for simultaneous determination of TEL and CHL. The mobile phase Acetonitrile: Toluene: Glacial acetic acid (7.5:2.5:0.05v/v/v) resulted in good resolution and sharp and symmetrical peaks of  $R_f$  0.26 ± 0.02 for TEL and 0.67 ± 0.02 for CHL. It was observed that pre-washing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 15 min (optimum chamber saturation time) ensured good reproducibility and peak shape of both the drugs. (Fig. 3)

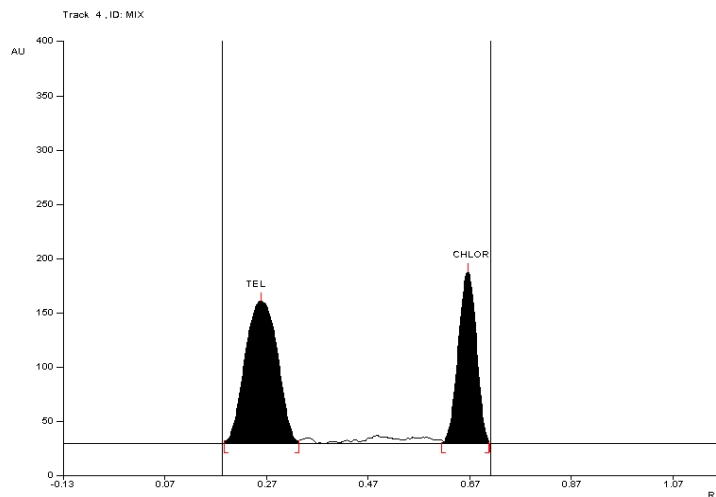


Fig. 3: HPTLC Chromatogram of Standard TEL and CHL in mixture

### Linearity

Linear regression data for the calibration plots revealed good linear relationships between area and concentration over the ranges 400-2400 ng/spot for TEL and 125-750 ng/spot for CHL. The linear equations for the calibration plots were  $y = 3.377x + 1356$  and  $y = 7.726x + 201.3$  with Regression ( $r^2$ ) being 0.995 and 0.995 for TEL and CHL, respectively (Fig. 4,5,6) (Table 1, 2 and 3).

### Precision

The precision of the method was expressed as relative standard deviation (RSD %). The %R.S.D. values for intra-day precision study and inter-day study listed in (Table 4 and 5) were <1.0%, confirming that the method was sufficiently precise.

### Accuracy

When the method was used for accuracy and subsequent analysis of both the drugs from the pharmaceutical dosage form, and spiked with 80, 100, and 120% of additional pure drug, the recovery was

found to be %99.95-100.11 for TEL and %99.81-100.41 for CHL (Table 6 and 7).

### Sensitivity

The LOD and LOQ were calculated by equation. The LOD and LOQ values were 9.049 and 27.422 ng/spot for TEL and 5.147 and 15.598 ng/spot for CHL.

### Specificity

The peak purity of TEL and CHL were assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e.,  $r(S, M) = 0.9996$  and  $r(M, E) = 0.9990$  for TEL and  $r(S, M) = 0.9984$  and  $r(M, E) = 0.9989$  for CHL. Good match was obtained between standard and sample spectra of TEL and CHL respectively. (Fig. 7)

### Repeatability

The % RSD for peak area values of TEL and CHL were found to be 0.1206 and 0.3388 respectively, as given in Table 8.

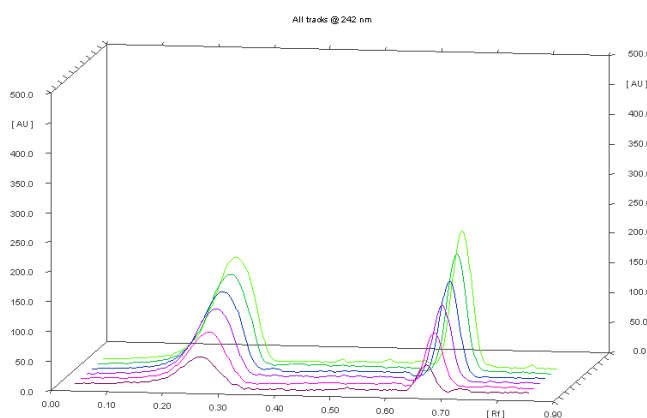


Fig. 4: 3D Representation of Densitogram for Calibration curve of TEL and CHL

Table 1: Result of Calibration readings for TEL

Concentration (ng/spot)	R <sub>f</sub>	Area Mean (n=6) ± SD	%RSD
400	0.25	2564.967±6.729	0.2623
800	0.26	3396.017±11.673	0.2921
1200	0.26	5574.767±10.142	0.1819
1600	0.26	6997.55±8.434	0.1205
2000	0.27	8108.433±9.03	0.1113
2400	0.27	9270.767±9.559	0.1031

Table 2: Result of Calibration readings for CHL

Concentration (ng/spot)	R <sub>f</sub>	Area Mean (n=6) ± SD	%RSD
125	0.66	991.5667±10.539	1.0629
250	0.66	2199.7±12.121	0.551
375	0.67	3249.483±12.844	0.3952
500	0.67	4143.667±14.057	0.3392
625	0.67	5029.783±11.72	0.233
750	0.67	5875.1±11.027	0.1877

Table 3: Statistical Data of TEL and CHL

Parameters	Results	
	TEL	CHL
Linear Range(ng/spot)	400-2400	125-750
Slope	3.377	7.726
Intercept	1356	201.3
Std. Deviation of Slope	0.00608	0.01269
Std. Deviation of Intercept	9.3594	10.7742
Limit of Detection(ng/spot)	9.05	5.15
Limit of Quantitation(ng/spot)	27.42	15.6
Regression Equation	$y = 3.377x + 1356$	$y = 7.726x + 201.3$
Co-Relation Co-Efficient (r)	0.99749	0.99749
Co-Efficient of Determination ( $r^2$ )	0.995	0.995

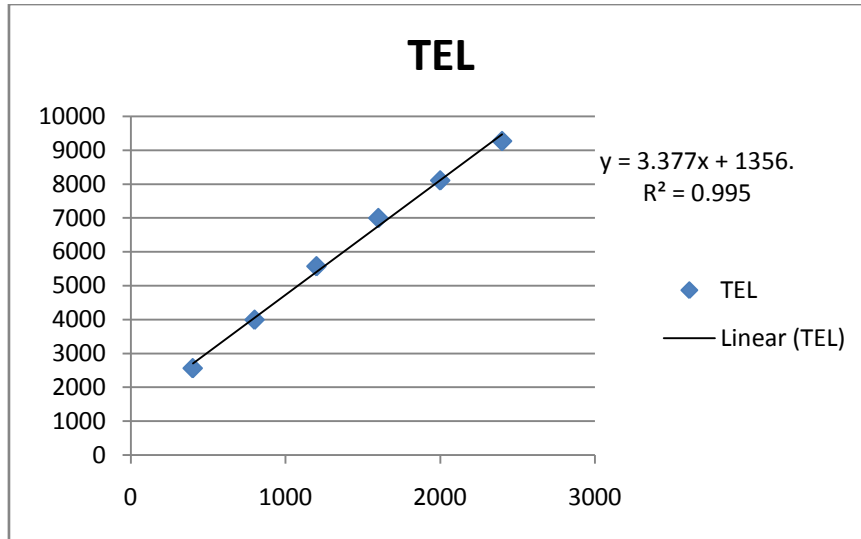


Fig. 5: Calibration curve of TEL in Methanol at 242 nm

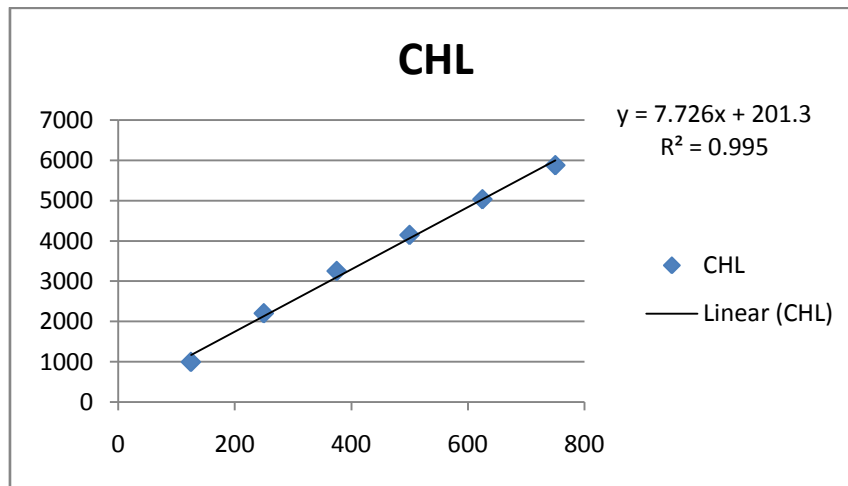


Fig. 6: Calibration curve of CHL in Methanol at 242 nm

Table 4: Intra-Day and Inter-Day study of TEL

Concentration (ng/spot)	Intra-Day Area Mean (n=3) ± SD	%RSD	Inter-Day Area Mean (n=3) ± SD	%RSD
800	3995.1±3.305	0.0827	3994.73±2.515	0.0629
1200	5575.166±3.625	0.065	5575.966±3.564	0.0639
1600	6999.567±3.711	0.053	6998.33±3.763	0.0537

Table 5: Intra-Day and Inter-Day study of CHL

Concentration (ng/spot)	Intra-Day Area Mean (n=3) ± SD	%RSD	Inter-Day Area Mean (n=3) ± SD	%RSD
250	2200.233±3.372	0.1533	2198.5±4.782	0.2175
375	3249.367±2.369	0.0729	3250.033±3.493	0.1075
500	4144.633±3.004	0.0724	4148.367±3.296	0.0795

Table 6: Determination of Accuracy for TEL

Concentration of Sample Taken (ng/spot)	Concentration of Pure API spiked (ng/spot)	Total Concentration (ng/spot)	Mean Total Concentration Found (n=3) (ng/spot)	%Recovery Mean (n=3)	%RSD
800	640	1440	1439.69	99.95	0.2087
	800	1600	1600.85	100.11	0.2441
	960	1760	1760.25	100.03	0.2268
Average				100.03	

Table 7: Determination of Accuracy for CHL

Concentration of Sample Taken (ng/spot)	Concentration of Pure API spiked (ng/spot)	Total Concentration (ng/spot)	Mean Total Concentration Found (n=3) (ng/spot)	%Recovery Mean (n=3)	%RSD
250	200	450	449.6159	99.81	0.7528
	250	500	501.0267	100.41	0.6213
	300	550	549.5944	99.86	0.7346
Average				100.026	

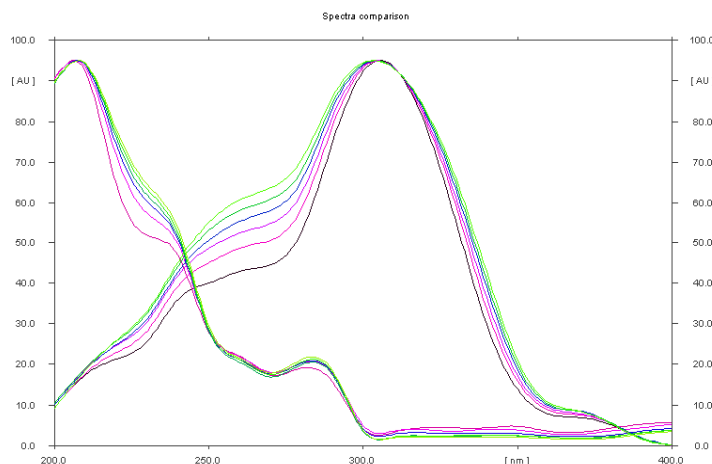


Fig. 7: UV Absorption (Reflectance Mode) of the corresponding spots for TEL and CHL

Table 8: Repeatability study of TEL and CHL

Concentration	TEL (1600ng/spot)	CHL (500ng/spot)
Area	7009 6991.7 7001.1 6985.2 7002.4 6996.1	4144.9 4135.7 4159.3 4161.5 4129.1 4131.6
Mean	6997.583	4143.683
± SD	8.439	14.037
%RSD	0.1206	0.3388

Table 9: Assay Result of Marketed Formulation

Parameters	Eritel CH-40 TAB	
	TEL	CHL
Actual Concentration (ng/spot)	1200	375
Concentration Obtained (ng/spot)	1204.56	374.44
%Purity	100.38	99.85
%RSD	0.5897	0.896
Limit[5]	90-110	92.5-107.5

Table 10: Validation Parameters

Summary of Validation Parameters	TEL	CHL
Recovery (%)	100.03	100.026
Repeatability (%RSD)	0.1206	0.3388
Precision (CV)		
Intra-day (n=3)	0.000669	0.000996
Inter-day (n=3)	0.000602	0.00135
Specificity	Specific	Specific
Selectivity	Selective	Selective

### Analysis of TEL and CHL in marketed formulation

When the Eritel- CH40 tablets were analyzed, TEL and CHL gave sharp and well defined peaks at  $R_f$   $0.26 \pm 0.02$  and  $0.67 \pm 0.02$ , respectively, when scanned at 242 nm. The results in Table 9 indicate that there was no interference from the excipients commonly present in the tablet formulation. The % purity was %100.38 for TEL and %99.85 for CHL.

### CONCLUSION

The developed HPTLC method is simple, precise, accurate and reproducible and can be used for simultaneous determination of TEL and CHL in pharmaceutical dosage forms. The method was validated as per International Conference on Harmonization (ICH) guidelines.

### ACKNOWLEDGEMENT

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