RP-HPLC METHOD DEVELOPMENT & VALIDATION FOR SIMULTANEOUS ESTIMATION OF CHLORTHALIDONE, METOPROLOL SUCCINATE AND TELMISARTAN IN TABLETS

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ABSTRACT: A simple, rapid, economical, precise and accurate RP-HPLC method simultaneous estimation of Chlorthalidone, metoprolol succinate and Telmisartan in tablets has been developed.

A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Chlorthalidone, Metoprolol succinate and Telmisartan in tablets has been developed. The separation was achieved by Hypersil BDS C_{18} (250mm x 4.6mm, 5μm) column and Buffer (0.5M potassium dihydrogen ortho phosphate, pH was adjusted with 1% orthophosphoric acid, pH3.0): methanol (50:50v/v) as a mobile phase at a flow rate of 1ml/min. detection was carried out at 215nm Retention time of Chlorthalidone- 6.690, Metoprolol – 8.813 and Telmisartan- 4.887minute. The method has been validated for linearity, accuracy and precision. Linearity observed for Chlorthalidone 3.12- 18.75 μg/ml, Metoprolol succinate 12.5- 75 μg/ml and Telmisartan 10-60μg/ml. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Chlorthalidone, metoprolol succinate and Telmisartan in Tablets.

KEYWORDS: Chlorthalidone, Metoprolol succinate, Telmisartan, RP-HPLC method, Validation.

INTRODUCTION

Chlorthalidone (2-chloro-5-(1-hydroxy-3-oxo-2*H*-isoindol-1yl) benzenesulfonamide) inhibits the reabsorption of sodium at the level of the distal convoluted tubule and thus chloride via inhibition of the Na/Cl symporter. Molecular formula of chlorthalidone is $C_{14}H_{11}CLN_2O_4S$ and molecular weight 338.8g/mol. Metoprolol succinate (l -(lsopropylamino)-3-[p-(2-methoxyethyl)phenoxy]- 2-propanol succinate (2:1)) Metoprolol is a cardio selective beta-1-adrenergic receptor inhibitor that competitively blocks beta1-receptors with minimal or no effects on beta-2 receptors at oral doses of less than 100 mg in adults. It decreases cardiac output by negative inotropic and chronotropic effects. Molecular weight of Metoprolol succinate is $(C_{15}H_{25}NO_3)_2 . C_4H_6O_4$. Telmisartan (4'. [[4-Methyl-6- {1 -methyl-2 -benzimidazolyl)-2 -propyll - benzimidazolyl] methyl]-2-biphenylcarboxyhc acid.) is interferes with the binding of angiotensin II to the angiotensin II AT_1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. As angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone, blockage of its effects results in decreases in systemic vascular resistance. Molecular formula of telmisartan is $C_{33}H_{30}N_4O_2$ and molecular weight is 513.6 g/mol. There is no HPLC method available for above combination of the afore mentioned drugs. The present research work, is aimed to carry out the reverse phase HPLC method for simultaneous estimation of Metoprolol, Telmisartan and Chlorthalidone to meet the ICH and various regulatory authority guidelines. The newly developed method was validated in accordance with the analytical parameter mentioned in the ICH guidelines.

MATERIAL AND METHOD

chlorthalidone, Metoprolol succinate and Telmisartan sample was kindly provided by molecular laboratories, Ahmedabad, India. All chemicals were at least of analytical grade were used. HPLC grade Methanol, water and acetonitrile procured from the final Limited, Gujarat, India and potassium di- hydrogen ortho phosphate and ortho- phosphoric acid (AR- Grade) were procured from the Ranbaxy chemicals, New Delhi, India.

INSTRUMENT

HPLC was performed with a Model: Agilent Technologies,1220 infinity, Pump: -LC-20 AT, Chromatographic separation was achieved using Hypersil BDS C_{18}, (250 mm × 4.6 mm, 5 μm) column. column Injector: 20μL fixed loop. Detector: SPD 20 A UV Detector. (Software: open lab control panel).

METHOD DEVELOPMENT

Preparation of standard solution

Chlorthalidone (12.5mg) was weight and Transferred to 100ml volumetric flask and volume was mad up to the 100ml with methanol, Metoprolol succinate (50 mg) was weight and Transferred to 100ml volumetric flask and volume was mad up to the 100ml with methanol and Telmisartan (40 mg) was weight and Transferred to 100ml volumetric flask and volume was mad up to the 100ml with methanol.

Preparation of working standard solution

One ml from Chlorthalidone, 1ml from Metoprolol succinate and 1ml from Telmisartan standard stock solution transferred to 10 ml volumetric flask and volume was made up to the mark by mobile phase.

Phosphate buffer preparation:

potassium dihydrogen orthophosphate (6.8gm) were weight and transfer into the 1000ml beaker. Add 800ml water were added and dissolved. volume was made up with water and pH was adjusted by 1% Orthophosphoric acid (pH 0.3) solution. (0.5M potassium dihydrogen ortho phosphate, pH – 3.0 buffer)

pharmaceutical formulation:

Twenty tablets were weighed individually and average weight was found. Tablet Powder equivalent to 12.5mg of Chlorthalidone, 50mg of Metoprolol succinate and 40 mg of Telmisartan was transferred to 100 ml volumetric flask, and add 60 ml of mobile phase and shake for 15 minutes and sonicate for 5 minutes. Made up the volume to the mark with mobile phase. The solution was filtered through Whatman filter paper no-01and first few drops of filtrate were discarded. One ml of this solution was diluted to 10 ml with mobile phase.

Selection of wavelength:

Standard solution of 12.5μg/ml Chlorthalidone, 50μg/ml of Metoprolol succinate and 40μg/ml of Telmisartan in methanol were scanned between 200-400nm using UV- Visible spectrophotometer. Both three solution were scanned between 200-400nm. Wavelength was selected from the overlay spectra of above solution.
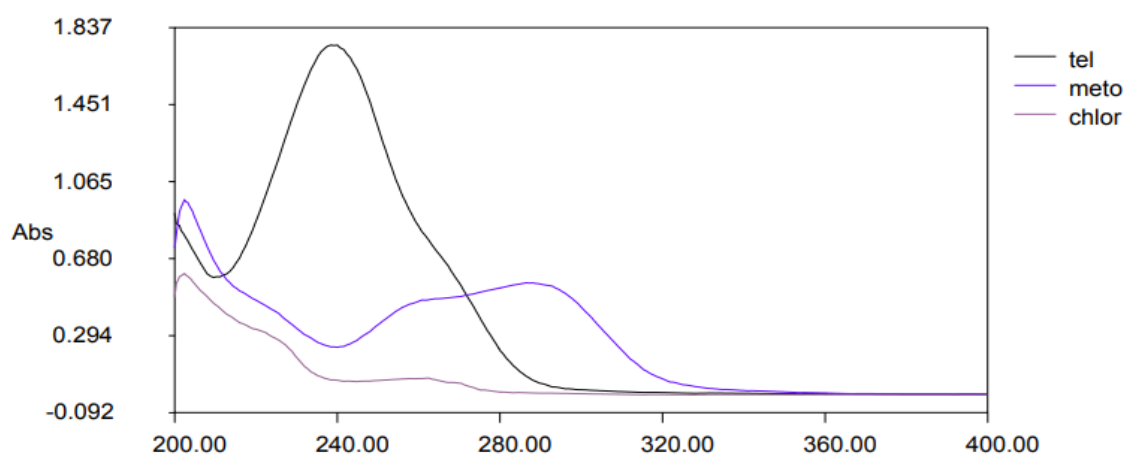

Figure 1: UV Spectra of Chlorthalidone, metoprolol succinate and Telmisartan (215nm Selected)

Mobile Phase selection:

Mobile Phase was selected based on the review of literature. Various mobile phases were tried. Trial contains various mobile phases which consisted of Methanol, Water, Buffers in different proportions with various pH and different volumes at different flow rate were tried. On the basis of various trials, the mixture of **KH2PO4 Buffer, pH 3.0: Methanol (50:50v/v)**

RESULT AND DISCUSSION

Optimize Chromatographic Condition:

1ml/min flow rate, proved to be better than the other in terms of resolution, peak shape and shorter retention time.

- ✓ **Mode of Elusion:** Isocratic

- ✓ **Column**: C$_{18}$ (25 cm × 0.46 cm) Hypersil BDS

- ✓ **Mobile Phase**: Buffer (pH 3.0): Methanol (50:50v/v)

- ✓ **Flow Rate**: 1.0 ml/min

- ✓ **Detection Wavelength**: 215 nm

- ✓ **Run time**: 20 min

- ✓ **Injection volume**: 20.0μl

METHOD VALIDATION

The method was validated with respect to linearity, limit of detection, limit of quantification, precision, accuracy, recovery and robustness.

System suitability

It is an integral part of chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. The system suitability of the system was studied by performing the experiment and looking for change in separation, retention times and asymmetry of the peaks. The resolution , areas, retention time, theoretical plates values and peak asymmetry were calculated. Result are obtained are given in table 1.

Table 1: System suitability test

Parameters	Chlorthalidone	Metoprolol succinate	Telmisartan
Retention Time	6.690	8.813	4.887
Theoretical Plates	7944	3447	7441
Tailing factor	1.268	1.276	1.387
Resolution	6.846	4.715	-

Linearity

The linearity for Chlorthalidone, Metoprolol succinate and Telmisartan were assessed by analysis of combined standard solution in range of 3.12-18.75 μg/ml,12.5-75 μg/ml and 10-60 μg/ml respectively. Calibration curve of the area was plotted and found out correlation co-efficient and regression line equation for Chlorthalidone, Metoprolol succinate and Telmisartan. Each response was an average of five determinations.

Table 2: Linearity results

Drug	Linearity range	Correlation coefficient
Chlorthalidone	3.12-18.75 μg/ml,	0.999
Metoprolol succinate	12.5-75 μg/ml	0.999
Telmisartan	10-60 μg/ml	0.999
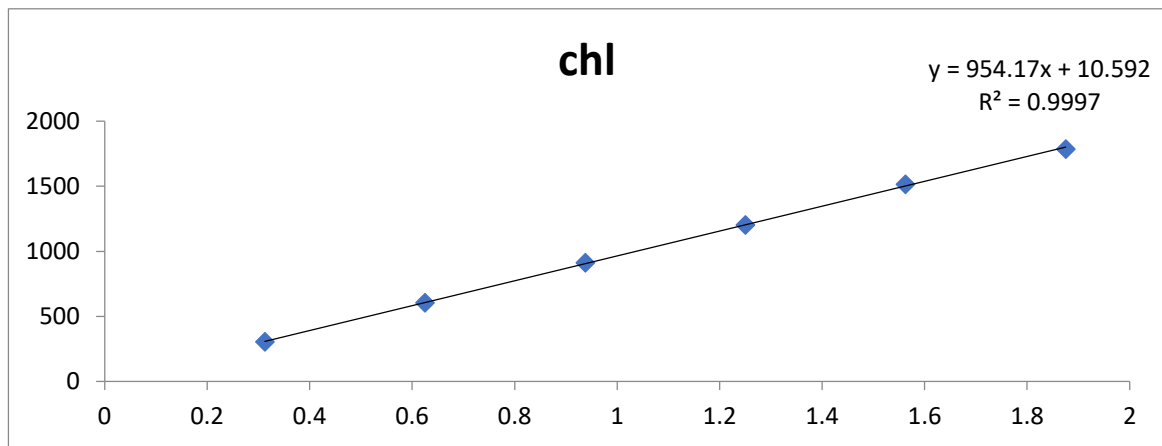
Figure 5.31: Calibration Curve of Chlorthalidone

$y = 954.17x + 10.592$
$R^2 = 0.9997$
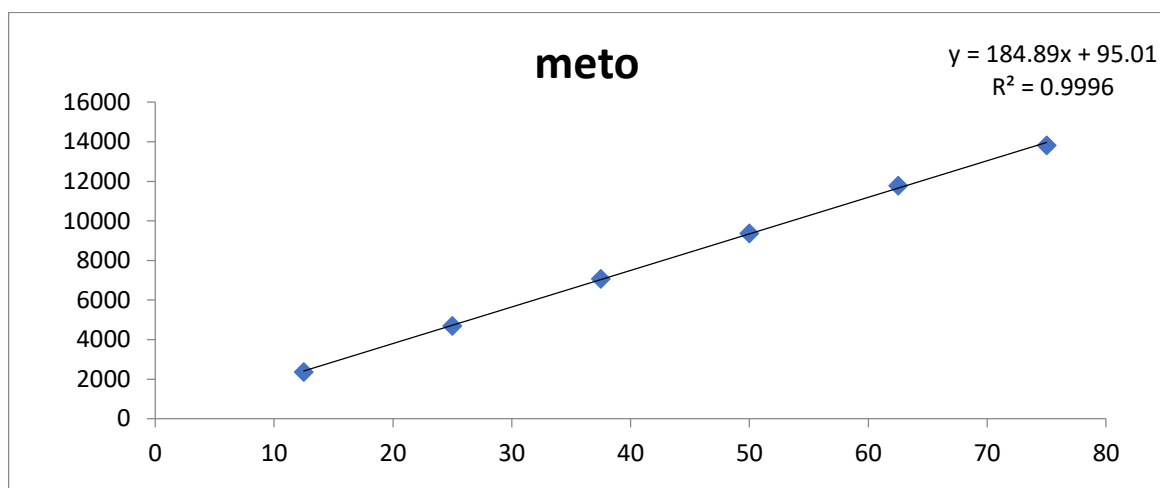
Figure 5.32: Calibration Curve of Metoprolol succinate

$y = 184.89x + 95.01$
$R^2 = 0.9996$
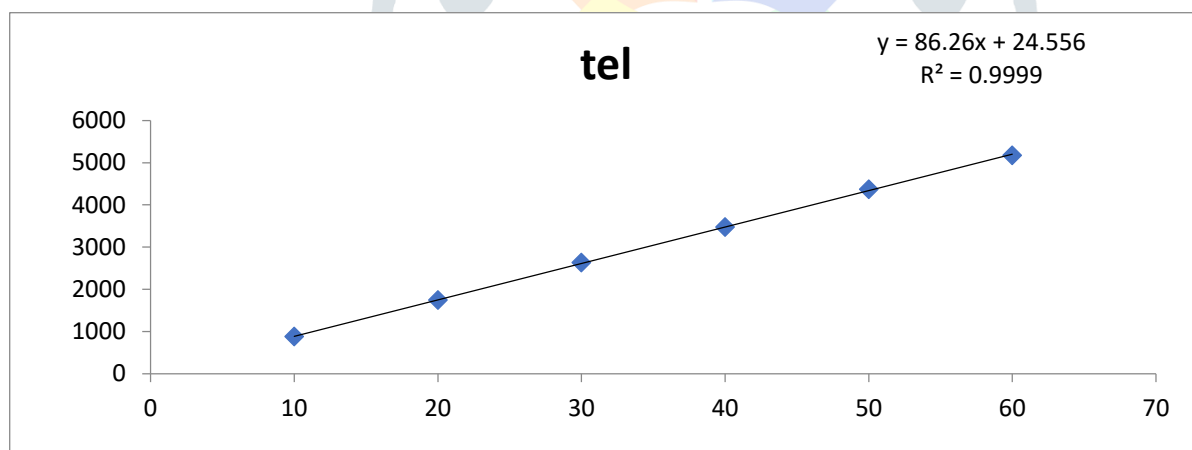
Figure 5.33: Calibration Curve of Telmisartan

$y = 86.26x + 24.556$
$R^2 = 0.9999$

PRECISION

Results should be expressed as Relative standard deviation (RSD) or coefficient of variance.

(A) Repeatability

The data for repeatability of peak area measurement for Chlorthalidone (12.5µg/ml), Metoprolol succinate (50µg/ml) and Telmisartan (40µg/ml) based on six measurements of same solution of Chlorthalidone (12.5µg/ml), Metoprolol succinate (50µg/ml) and Telmisartan (40µg/ml) and % R.S.D. was calculated.
% RSD of Chlorthalidone, Metoprolol succinate and Telmisartan was found to be 0.764, 0.915 and 0.784 respectively.

(B) Intraday precision

Standard solution containing Chlorthalidone (3.12,12.5 18.75µg/ml), Metoprolol succinate (12.5,50, 75µg/ml) and Telmisartan (10, 40, 60µg/ml) were analyzed three times on the same day and % R.S.D was calculated.

The % R.S.D. for intraday precision was found 0.289- 0.435 for chlorthalidone, 0.343- 1.165 for Metoprolol succinate and 0.301- 0.481 for Telmisartan.

(C) Inter day precision

Standard solution containing Chlorthalidone (3.12,12.5, 18.75μg/ml), Metoprolol succinate (12.5,50, 75μg/ml) and Telmisartan (10, 40, 60μg/ml) were analyzed three times on the different day and % R.S.D was calculated.

The % R.S.D. for inter day precision was found 0.699- 0.882 for chlorthalidone, 0.786- 1.299 for Metoprolol succinate and 0.529- 0.953 for Telmisartan.

Table 3: Precision result

Drug	Intra day precision (%RSD)	Inter day precision (% RSD)
Chlorthalidone	0289- 0.435	0.699- 0.882
Metoprolol succinate	0.343- 1.165	0.786- 1.299
Telmisartan	0.301- 0.481	0.529- 0.953

LOD & LOQ

The LOD was estimated from the set of 3 calibration curves used to determination method linearity. The LOD may be calculated as,

$LOD = 3.3 \times (SD/Slope)$

Where,

SD= Standard deviation of Y-intercepts of 3 calibration curves. Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine method linearity. The LOQ may be calculated as,

$LOQ = 10 \times (SD/Slope)$

Where, SD = Standard deviation of Y-intercepts of 3 calibration curves.

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts

Table 4: Result for LOD and LOQ

Parameter	Chlorthalidone	Metoprolol succinate	Telmisartan
LOD	0.2819μg/ml	7.2120μg/ml	1.0264μg/ml
LOQ	0.8543μg/ml	21.8547μg/ml	3.1105μg/ml

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are in table 5.26, 5.27 and 15.28. % recovery for Chlorthalidone was 100.39- 101.12%, for Metoprolol succinate 99.00-101.4 % and for Telmisartan, it was found to be in range of 100.56- 101.37%.

Table 5: Result of Accuracy

Drug	Amt. of drug (U=μg/ml)	Amount of drug added (μ/ml)	Amt. recovered Mean (μ/ml)	Mean % recovery $\pm$ S.D. (n=3)	Mean % RSD
Chlorthalidone	6.25	5	5.03	100.73$\pm$ 0.28	0.28
	6.25	6.25	6.32	101.12$\pm$ 0.75	0.0.74
	6.25	7.5	7.52	100.39$\pm$ 0.82	0.82
Metoprolol succinate	25	20	19.80	99.00$\pm$1.51	1.53
	25	25	25.36	101.46$\pm$1.21	1.19
	25	30	30.17	100.58$\pm$ 0.58	0.57
Telmisartan	20	16	16.22	101.37$\pm$1.64	1.61

20	20	20.23	101.19 ± 1.14	1.13
20	24	24.13	100.56 ± 0.61	0.61

ROBUSTNESS

Following parameters were changed one by one and their effect was Observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed ($\pm$ 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of Mobile phase was changed ($\pm$ 0.2) 3.2 and 2.8
3. Ratio of Mobile phase was changed ($\pm$2) Buffer: Methanol (52:48) and Buffer: Methanol (48:52)

Table 6: Robustness data for Chlorthalidone

Drug	Area at Flow Rate (+0.2 ml/min)	Area at Flow Rate (-0.2 ml/min)	Area at Moblie phase (+2)	Area at Moblie phase (-2)	Area at pH (+0.2)	Area at pH (-0.2)
Chlorthalidone	1183.122	1238.251	1196.158	1214.225	1210.649	1185.720
Metoprolol succinate	9159.076	9633.501	9292.329	9426.807	9374.892	9189.901
Telmisartan	3402.426	3575.118	3440.427	3495.876	3487.341	3412.752

ASSAY

Applicability of the proposed method was tested by analysing the commercially available Tablet formulation Med- xl 3D. The results are shown in table 5.34.

Table 5.34 Analysis of marked formulation

Tablet	Label Claim			Assay (% of label claim) Mean $\pm$ S.D.		
	Chlorthalidone	Metoprolol succinate	Telmisartan	%Chlorthalidone	%Metoprolol succinate	%Telmisartan
Med-xl 3D	12.5mg	50mg	40mg	96.80 ± 0.37	97 ± 0.48	96.89 ± 0.39

The assay results were comparable to labelled value of each drug in Tablet dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

CONCLUSION

A reverse phase high performance liquid chromatography method was developed for the simultaneous estimation of chlorthalidone, metoprolol succinate and telmisartan in tablets. The fine separation with better resolution achieved by C_{18} (250mm x 4.6mm, 5µm) column and Buffer (0.5M potassium dihydrogen ortho phosphate, pH was adjusted with 1% orthophosphoric acid, pH3.0): methanol (50:50v/v) as a mobile phase at a flow rate of 1ml/min. Detection was carried out at 215nm. The Retention time of Chlorthalidone- 6.690, Metoprolol – 8.813 and Telmisartan- 4.887minute. The method has been validated for its all parameters like – linearity, precision, accuracy, and robustness. Linearity observed for Chlorthalidone 3.12- 18.75 µg/ml, Metoprolol succinate

12.5- 75 µg/ml and Telmisartan 10-60µg/ml. The developed method found precise and accurate for simultaneous estimation of Chlorthalidone, metoprolol succinate and Telmisartan. The comparison sheet also describe that developed method is more precise and better in terms of mobile phase and runtime or resolution.

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