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Development and Validation of Analytical Methods for Simultaneous Estimation of Clonidine HCl and Chlorthalidone in Their Combined Dosage Forms

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ABSTRACT:

Reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Clonidine HCl and Chlorthalidone. In their Combined Dosage form has been developed. The separation was achieved by LC- 20 AT C18 (250mm x 4.6 mm x 2.6 µm) column and Buffer (pH 4.0)-Methanol (70:30) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 220 nm. Retention time of Clonidine HCl and Chlorthalidone were found to be 5.980 min and 4.150 min, respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Clonidine HCl 1.5-4.5 µg/ml and for Chlorthalidone 60-180 µg/ml. The percentage recoveries obtained for Clonidine HCl and Chlorthalidone were found to be in range of 99.56-101.02 and 99.11-100.88 respectively. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Clonidine HCl In Their Combined Dosage Form.

KEY-WORDS: Clonidine HCl, RP-HPLC, Mobile phase, Validation.

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INTRODUCTION:

Hypertension is defined as a sustained diastolic blood pressure greater than 90 mmHg by an elevated systolic blood pressure greater than 140 mmHg. Hypertension results from increased peripheral vascular smooth muscle tone and which leads to increased arteriolar resistance and reduced capacity of the venous system. Elevated blood pressure is an extremely common disease. Although many of these individuals have no symptoms, chronic hypertension either systolic or diastolic can lead to congestive heart failure, myocardial infarction, renal damage and cerebrovascular accidents.

CALSSIFICATION OF HYPERTENSION

Hypertension can be classified either as

- Essential (primary) hypertension
- Secondary hypertension

Essential hypertension is indicates that no specific medical cause can be found to explain a patient's condition.

Secondary hypertension indicates as high blood pressure is a result of (i.e., secondary to) another condition, such as kidney disease or tumors.

CAUSES OF HYPERTENSION

Essential (primary) Hypertension

By definition, essential hypertension has no identifiable cause. However, several risk factors are there, which are as follows:

- Obesity
- Salt sensitivity
- Insulin resistance
- Genetics
- Age
- Vitamin D deficiency
- Faulty lifestyle
- Smoking
- High consumption of Liquorices
- Tumors
- Renal hypertension
- Adrenal hypertension
- Cushing's syndrome

Secondary Hypertension

- Sleep apnea
- Contraction of the aorta
- Polycystic kidney disease
- Glucocorticoid remediable aldosteronism
- Pregnancy

Materials and methods

Materials

a) Instruments

- Analytical Weighing Balance
- Sonicator
- FT-IR spectrophotometer
- HPLC system

b) Glasswares

- Beaker
- Conical flask
- Measuring cylinder
- Petri dish
- Pipette
- Volumetric flask

c) Chemicals

- Marketed formulation of **Clonidine hydrochloride and Chlorthalidone**
- Solvents supposed to be use: Methanol, Acetonitrile, Ethanol ,Water,HCl etc.

d) Methods

- Chromatographic method

Methods

Working Standard and Sample preparation

(A) Clonidine standard stock solution: (30 µg/mL)

A 3 mg of Clonidine was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with methanol.

(B) Chlorthalidone standard stock solution: (1200 µg/mL)

A 12 mg of Chlorthalidone was weighed and transferred to a 10 mL volumetric flask. volume was made up to the mark with methanol.

(C) Preparation of standard solution of binary mixtures of Clonidine (3 µg/mL) and Chlorthalidone (120 µg/mL)

Take 1 mL from the Clonidine stock solution and 1mL from Chlorthalidone stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

METHOD VALIDATION

Chromatographic conditions and System Suitability Parameters:

✓ Model: Thermosepration.
✓ Column: C_{18} (25 cm × 0.46 cm) Hypersil BDS
✓ Injector: 20µL fixed loop.
✓ Detector: SPD 20 A UV Detector
✓ Software: DATA ACE
✓ Analytical balance: Electronic analytical balance (shimadzu)
✓ Corning volumetric flasks and pipettes

Mobile Phase: PHOSPHATE BUFFER:METHANOL(70:30)

System Suitability Parameters:

Retention time: CLONIDINE- 5.980 MINS ,CHLORTHALIDONE-4.150 MINS.

Asymmetry: CLONIDINE- 1.395 ,CHLORTHALIDONE-1.259

Theoretical plates: CLONIDINE- 7426 ,CHLORTHALIDONE-7428

Linearity and Range (n=3):

The linearity for Clonidine and Chlorthalidone were assessed by analysis of combined standard solution in range of 1.5-4.5 µg/ml and 60-180 µg/ml respectively, 5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Clonidine(30 µg/ml) and Chlorthalidone(1200 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 1.5,2.25,3,3.75 and 4.5 µg/ml and 60,90,120,150,180 µg/ml for Clonidine and Chlorthalidone respectively

In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

Precision

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

A. Repeatability

Standard solution containing Clonidine (3µg/ml) and Chlorthalidone (120µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated.

B. Intra-day precision

Standard solution containing (1.5,3,4.5 µg/ml) of Clonidine and (60,120,180 µg/ml) of Chlorthalidone were analyzed three times on the same day and % R.S.D was calculated.

C. Inter-day precision

Standard solution containing (1.5,3,4.5 µg/ml) of Clonidine and (60,120,180 µg/ml) of Chlorthalidone were analyzed three times on the different day and % R.S.D was calculated.

Accuracy

- ✓ **For Clonidine**

3 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 220 nm. The amount of Clonidine was calculated at each level and % recoveries were computed.

- ✓ **For Chlorthalidone**

120 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 220 nm. The amount of Chlorthalidone was calculated at each level and % recoveries were computed.

LOD and 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 × (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 × (SD/Slope)

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

Slope = Mean slope of the 3 calibration curves.

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 (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.

2. pH of Mobile phase was changed (± 0.2) 4.2 and 3.8.

3.Ratio of Mobile phase was changed(±2) Buffer:Methanol (68:32) and Buffer:Methanol (72:28)

RESULT AND DISCUSSION

VALIDATION PARAMETER

Linearity and Range

The linearity for Clonidine and Chlorthalidone were assessed by analysis of combined standard solution in range of 1.5-4.5 µg/ml and 60-180 µg/ml respectively. Correlation co-efficient for calibration curve Clonidine and Chlorthalidone was found to be 0.995 and 0.999.The regression line equation for Clonidine and Chlorthalidone are as following:

For Clonidine: **y = 199.3x - 31.08** and For Chlorthalidone : **y = 46.87x - 54**

For Clonidine: **y = 199.3x - 31.08** and For Chlorthalidone : **y = 46.87x - 54.64**

Table 1 : Linearity data for Clonidine.

Sr. No	Concentration (µg/ml)	Area
1	1.5	279.041
2	2.25	412.416
3	3	562.641
4	3.75	696.017
5	4.5	884.838

Table 2 : Linearity data for Chlorthalidone

Sr. No	Concentration (µg/ml)	Area
1	60	2785.389
2	90	4111.136
3	120	5619.841
4	150	6925.868
5	180	8409.18

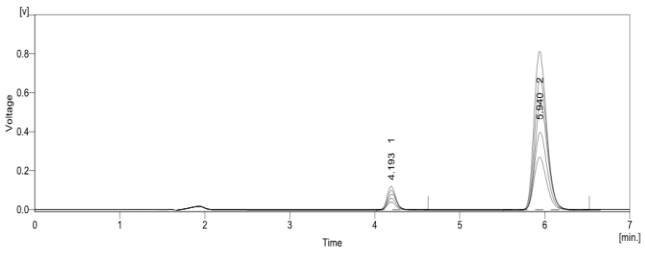

Figure 1: Overlay chromatogram of different concentrations of binary mixtures of Chlorthalidone and Clonidine

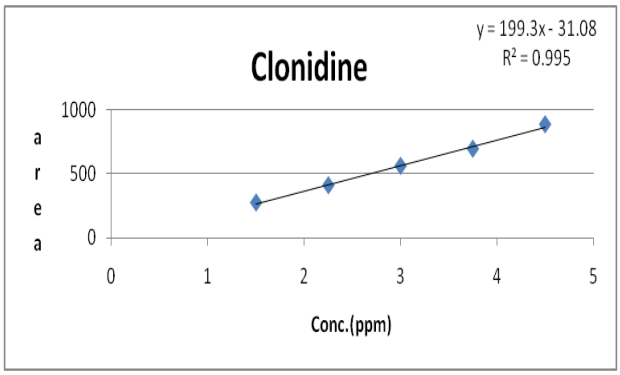

Figure 2: Calibration Curve of Clonidine (1.5-4.5 µg/ml).

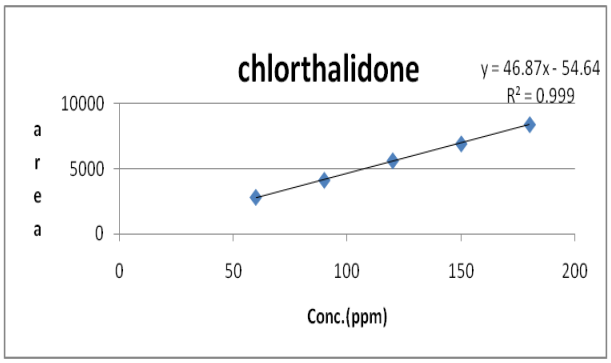

Figure 3: Calibration Curve of Chlorthalidone (60-180 µg/ml).

Precision

Repeatability

The data for repeatability of peak area measurement for Chlorthalidone and Clonidine, based on six measurements of same solution of Chlorthalidone and Clonidine are depicted in table 4 & 3 The % RSD for Chlorthalidone and Clonidine was found to be 0.229 and 0.210 respectively.

Table 3: Repeatability data for Clonidine.

Clonidine			
Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
3	561.236 562.325 563.378 564.474 561.666 562.881	566.620±1.182	0.210

Table 4. repeatability data for Chlorthalidone

Chlorthalidone			
Conc (µg/ml)	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
120	5597.744 5608.03 5620.115 5631.386 5603.36 5601.798	5610.406 ±12.838	0.229

II. Intraday precision

The data for intraday precision for Chlorthalidone and Clonidine is shown in table 5. The % R.S.D. for Intraday precision was found to be 0.118-0.926. for Clonidine and 0.215-0.284 for Chlorthalidone.

Table 5 : Intraday precision data for estimation of Chlorthalidone and Clonidine

		Clonidine			Chlorthalidone	
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	1.5	285.228 ± 0.388	0.118	60	2765.938± 5.954	0.215
2	3	559.219± 1.646	0.294	120	5579.568± 13.212	0.237
3	4.5	833.804± 7.725	0.926	180	8347.806± 23.703	0.284

Interday precision

The data for intraday precision for Chlorthalidone and Clonidine is shown in table 6. The % R.S.D. for interday precision was found to be 0.554-1.586 for Clonidine and 0.319-0.497 for Chlorthalidone.

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in table 7 and 8. Percentage recovery for Clonidine was 99.56-101.02 %, while for Chlorthalidone, it was found to be in range of 99.11-100.88%.

Table 6: Interday precision data for estimation of Chlorthalidone and Clonidine

		Clonidine			Chlorthalidone	
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	1.5	281.243± 3.983	1.416	60	2758.839± 8.820	0.319
2	3	558.295± 3.092	0.554	120	5569.904± 27.669	0.497
3	4.5	829.566± 13.158	1.586	180	8338.668± 34.387	0.412

Table 7: Recovery data for Clonidine

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	1.5	1.2	1.213	101.111	100.944 ± 0.905
2		1.5	1.2	1.221	101.754	
3		1.5	1.2	1.200	99.967	
4	100 %	1.5	1.5	1.496	99.702	100.161 ± 0.814
5		1.5	1.5	1.495	99.681	
6		1.5	1.5	1.517	101.101	
7	120 %	1.5	1.8	1.808	100.445	100.740 ± 0.721
8		1.5	1.8	1.828	101.561	
9		1.5	1.8	1.804	100.212	

Table 8 : Recovery data for Chlorthalidone

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	60	48	48.502	101.045	101.255 ± 0.367
2		60	48	48.806	101.679	
3		60	48	48.499	101.040	

4	100 %	60	60	59.718	99.530	99.754 ± 1.075
5		60	60	59.285	98.808	
6		60	60	60.554	100.923	
7	120 %	60	72	72.189	100.263	100.392 ± 0.201
8		60	72	72.208	100.290	
9		60	72	72.449	100.624	

LOD and LOQ

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 9: Limit of Detection data for Clonidine and Chlorthalidone

Limit of Detection :

Clonidine	Chlorthalidone
LOD = 3.3 x (SD / Slope)	LOD = 3.3 x (SD / Slope)
= 3.3 x (17.712/199.3)	= 3.3 x (55.617/46.870)
= 0.293 µg/ml	= 3.916 µg/ml

Limit of Quantitation :

Table 10: Limit of Quantitation data for Clonidine and Chlorthalidone

Clonidine	Chlorthalidone
LOQ = 10 x (SD / Slope)	LOQ = 10 x (SD / Slope)
= 10 x (17.712/199.3)	= 10 x (55.617/46.870)
= 0.888 µg/ml	= 11.866 µg/ml

Robustness

The effect of changes was found to be within the acceptance criteria as shown in table 11 and table 12. The % RSD should Be less than 2%.

Table 11: Robustness data for Clonidine

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase (-2)	Area at Mobile phase(+2)
1	582.766	547.494	577.747	533.746	575.450	537.362
2	584.813	551.151	579.654	539.719	578.492	550.062
3	583.440	554.458	582.766	543.159	581.592	553.353
% R.S.D	0.178	0.632	0.436	0.883	0.531	1.544

Table 12: Robustness data for Chlorthalidone.

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase(-2)	Area at Mobile phase (+2)
1	5805.438	5440.577	5747.810	5353.567	5708.403	5444.733
2	5827.767	5489.478	5770.812	5376.229	5759.250	5478.476
3	5849.760	5523.227	5805.438	5409.855	5793.813	5512.179

% R.S .D	0.380	0.757	0.502	0.526	0.746	0.615

Analysis of marketed formulation by developed method.

Applicability of the proposed method was tested by analyzing the commercially available Tablet formulation CLORPRES .The results are shown in table 13

Table 13 : Analysis of marketed formulation

Tablet	mg/Tablet powder		Assay (% of label claim*) Mean ± S. D.	
	Clonidine	Chlorthalidone	% Clonidine	% Chlorthalidone
Clorpres	0.3	12	101.506±10 0.689 2.218124	100.689 ± 0.403

The assay results were comparable to labeled 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

- The proposed RP-HPLC method is simple, precise, accurate, economic and rapid for the determination of Clonidine and Chlorthalidone in bulk drug and in combined Tablet dosage form.
- Analysis of authentic sample containing Clonidine and Chlorthalidone showed no interference from the common additives and excipients.
- It can be successfully adopted for routine quality control analysis of Clonidine and Chlorthalidone in combined Tablet dosage form without any interference from common excipients and impurity.

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