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Research

ESTIMATION OF CLONIDINE HCL AND CHLORTHALIDONE BY RP-HPLC METHOD

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Abstract

Background: A simple, accurate and precise HPLC method for simultaneous determination of Clonidine HCl and Chlorthalidone in pure and tablet dosage form has been developed.

Aim: To develop and validate analytical method for simultaneous estimation of Clonidine HCl and Chlorthalidone in pharmaceutical formulation by RP-HPLC.

Materials and Methods: HPLC of Waters (Model: Alliance 2695) with Phenomenex Luna C18 (4.6 mm I.D. × 250 mm, 5 µm) column was used for chromatographic separation. It contains waters injector and PDA Detector (Deuterium). Mobile phase consists of Methanol: Water (65:35% v/v) and flow rate adjusted was 1ml/min. Wavelength selected for detection was 220nm and injection volume was 10 µl.

Results and discussion: By using the developed method, retention time of Clonidine HCl and Chlorthalidone was found to be 3.2min and 5.4min respectively. The method has been validated for linearity, accuracy and precision. Linearity of Clonidine HCl and Chlorthalidone were in the range of 75–375µg/ml and 15–75µg/ml respectively. The percentage recoveries obtained for Clonidine HCl and Chlorthalidone were found to be in range of 99.3 – 99.6%. LOD and LOQ were found to be 12.5µg/ml and 38.1µg/ml for Clonidine HCl 3.7and 11.4µg/ml for Chlorthalidone.

Conclusion: The developed HPLC method offers several advantages such as rapidity, usage of simple mobile phase and easy sample preparation steps. Further, improved sensitivity makes it specific and reliable for its intended use. Hence, this method can be applied for the analysis of pure drug and pharmaceutical dosage forms.

From the present study it can be concluded that the proposed method is simple, sensitive, precise, specific, accurate and reproducible. Results of validation parameters demonstrated that the analytical procedure is suitable for its intended purpose and meets the criteria defined in ICH Q2R1.

Keywords: Clonidine HCl, Chlorthalidone, Simultaneous Estimation, RP-HPLC

INTRODUCTION

Analytic method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products.

Method development:

Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the “process of demonstrating that analytical procedures are suitable for their intended use” [1-2]. Understanding of the physical and chemical characteristics of drug allows one to select the most appropriate high performance liquid chromatography method development from the available vast literature. Information concerning the sample, for example, molecular mass, structure and functionality, pKa values and UV spectra, solubility of compound should be compiled. The requirement of removal of insoluble impurities by filtration, centrifugation, dilution or concentration to control the concentration, extraction (liquid or solid phase), derivatization for detection etc. should be checked. For pure compound, the sample solubility should be identified whether it is organic solvent soluble or water soluble, as this helps to select the best mobile phase and column to be used in HPLC method development.

Method development in HPLC can be laborious and time consuming. Chromatographers may spend many hours trying to optimize a separation on a column to accomplish the goals. Even among reversed phase columns, there is astonishing diversity, owing to differences in both base silica and bonded phase characteristics. Many of these show unique selectivity. What is needed is a more informed decision making process for column selection that may be used before the chromatographer enters the laboratory. The method of column selection presented here involves a minimal investment in time initially, with the potential of saving many hours in the laboratory.

Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods that support safety and characterization studies or evaluations of drug performance are also to be evaluated. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate the potential degradation of the API in the presence of formulation excipients [3, 4].

The three critical components for a HPLC method are: sample preparation (% organic, pH, shaking/sonication, sample size, sample age) analysis conditions (%organic, pH, flow rate, temperature, wavelength, and column age), and standardization (integration, wavelength, standard concentration, and response factor correction). During the preliminary method development stage, all individual components should be investigated before the final method optimization. This gives the scientist a chance to critically evaluate the method performance in each component and streamline the final method optimization [5]. The percentage of time spent on each stage is proposed to ensure the scientist will allocate sufficient time to different steps. In this approach, the three critical components for a HPLC method (sample preparation, HPLC analysis and standardization) will first be investigated individually [6-8].

The degraded drug samples obtained are subjected to preliminary chromatographic separation to study the number and types of degradation products formed under various conditions [9]. Scouting experiments are run and then conditions are chosen for further optimization [10]. Resolving power, specificity, and speed are key chromatographic method attributes to keep in mind during method development [11]. Selectivity can be manipulated by combination of different factors like solvent composition, type of stationary phase, mobile phase, buffers and pH. Changing solvents and stationary phases are the most comfortable approaches to achieve the separation. The proper range of pH is an important tool for separation of ionizable compounds. Acidic compounds are retained at low pH while basic compounds are more retained at higher pH. The neutral compounds remain unaffected. The pH range 4-8 is not generally employed because slight change in pH in this range would result in a dramatic shift in retention time. However, by operating at pH extremes (2-4 or 8-10), not only is there a 10-30 fold difference in retention time that can be exploited in method development but also the method can be made more robust which is a desirable outcome with validation in minutes [12,13]. Various steps for HPLC method development are given below.

EXPERIMENTAL WORK

INSTRUMENTS USED

Instruments and Glass wares	Model
HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
pH meter	LabIndia
Weighing machine	Sartorius
Volumetric flasks	Borosil
Pipettes and Burettes	Borosil
Beakers	Borosil
Digital ultra sonicator	Labman

CHEMICALS USED:

Clonidine (Pure)	Sura labs
Chlorthalidone (Pure)	Sura labs
Water and Methanol for HPLC	LICHROSOLV (MERCK)
Acetonitrile for HPLC	Merck

RESULTS AND DISCUSSION

Trail 4 (Optimized chromatogram):

Column : Phenomenex Luna C18 (4.6×250mm) 5µ

Column temperature : 35°C

Wavelength : 220nm

Mobile phase ratio : Methanol:Water (65:35 v/v)

Flow rate : 1ml/min

Injection volume : 10µl

Run time : 10minutes

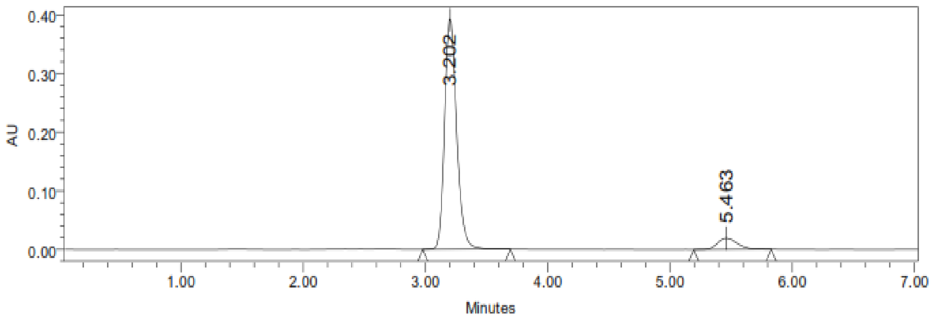


Figure: Optimized Chromatogram (Standard)

Table: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP
1	Clonidine HCl	3.202	2391746	39726	1.2	9028	
2	Chlorthalidon	5.463	194627	8497	1.1	7398	7.4

Optimized Chromatogram (Sample)

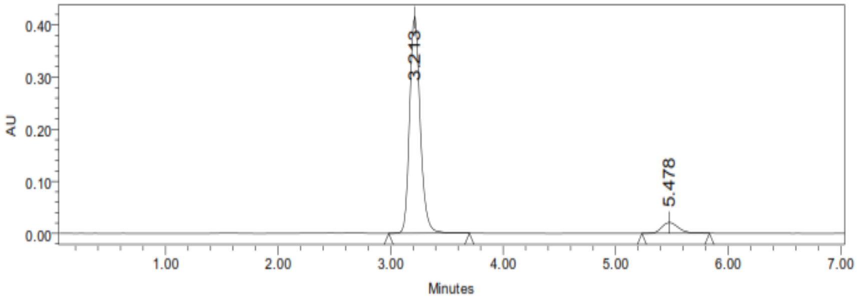


Table: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Clonidine HCl	3.213	2381649	391846	1.2	9472	
2	Chlorthalidone	5.478	191057	8104	1.1	8936	7.5

Acceptance criteria:

- Resolution between two drugs must be not less than 2
- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION

Blank:

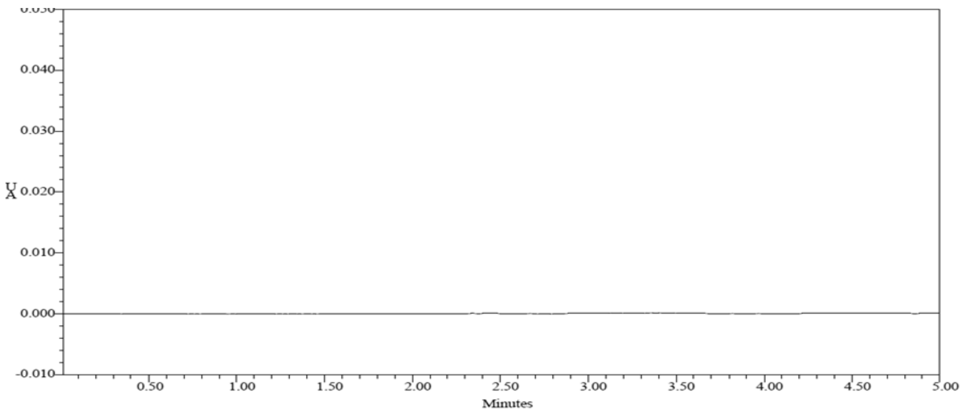


Fig: Chromatogram showing blank (mobile phase preparation)

System suitability:

Table: Results of system suitability for Clonidine HCl

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Clonidine HCl	3.200	2391746	394171	8952	1.2
2	Clonidine HCl	3.248	2391647	381946	9561	1.2
3	Clonidine HCl	3.299	2381647	391746	6572	1.2
4	Clonidine HCl	3.297	2385631	386562	6452	1.2
5	Clonidine HCl	3.297	2385635	389164	7452	1.2
Mean			2387261			
Std. Dev.			4363.771			
% RSD			0.182794			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table: Results of system suitability for Chlorthalidone

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Chlorthalidone	5.413	198362	7917	5272	1.1
2	Chlorthalidone	5.484	197486	7486	6291	1.1

3	Chlorthalidone	5.405	198354	7859	6184	1.1
4	Chlorthalidone	5.405	197352	7926	7145	1.1
5	Chlorthalidone	5.409	198453	7946	6946	1.1
Mean			198001.4			
Std. Dev.			535.1774			
% RSD			0.27029			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Clonidine HCl and Chlorthalidone in drug product.

Assay (Standard) :

Table: Peak results for assay standard

Clonidine HCl

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Clonidine HCl	3.211	2397162	397161	1.2	9472
2	Clonidine HCl	3.222	2394721	389173	1.2	9745
3	Clonidine HCl	3.254	2389461	391723	1.2	8917

Chlorthalidone

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Chlorthalidone	5.414	198462	7811	1.1	8492	7.49
2	Chlorthalidone	5.453	198472	8193	1.1	8916	7.52

3	Chlorthalidone	5.424	198735	7972	1.1	9372	7.44

Assay (Sample):
Table: Peak results for Assay sample

Clonidine HCl

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Clonidine HCl	3.297	2391741	381612	1.2	9472
2	Clonidine HCl	3.294	2389166	391746	1.2	8927
3	Clonidine HCl	3.295	2361731	381634	1.2	9017

Chlorthalidone

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Chlorthalidone	5.435	198641	8174	1.1	9284	7.18
2	Chlorthalidone	5.417	196547	8942	1.1	8974	7.44
3	Chlorthalidone	5.434	194027	7294	1.1	9017	7.38

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Clonidine HCl and Chlorthalidone in pharmaceutical dosage form was found to be 99.2%.

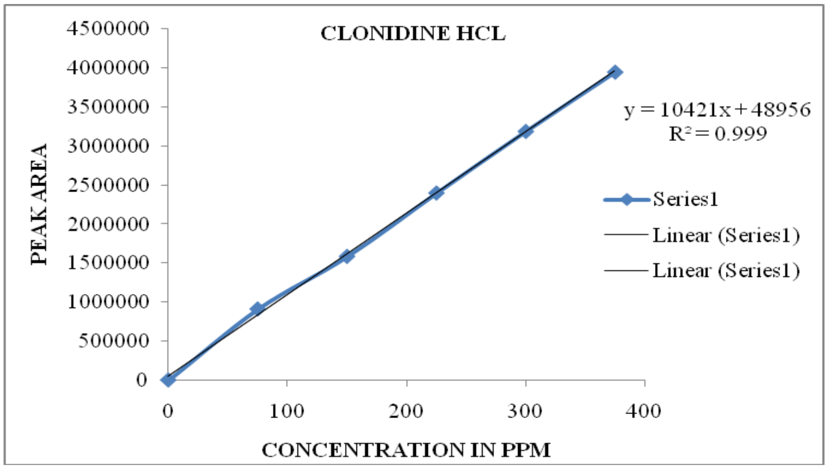
LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Clonidine HCl

Concentration µg/ml	Average Peak Area
75	909889
150	1583641
225	2395378

300	3185089
375	3943725



LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Clonidine HCl is a straight line.

Y = mx + c

Slope (m) =10421

Intercept (c) = 48956

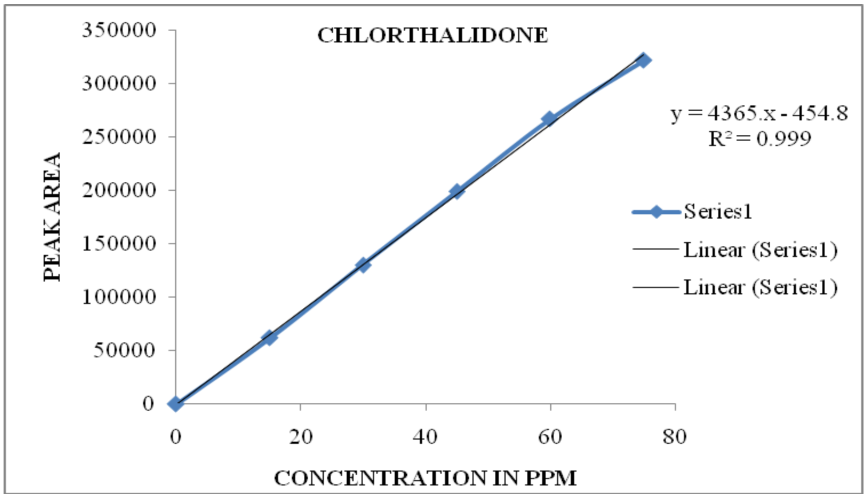
Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 48956. These values meet the validation criteria.

Chlorthalidone

Concentration µg/ml	**Average Peak Area**
15	61953
30	130213
45	198697
60	267002
75	321658



LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Chlorthalidone is a straight line.

Y = mx + c

Slope (m) = 4365

Intercept (c) = 454.8

Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 454.8. These values meet the validation criteria.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table: Results of repeatability for Clonidine HCl:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Clonidine HCl	3.213	2397164	381741	8155	1.2
2	Clonidine HCl	3.253	2391741	371742	9174	1.2
3	Clonidine HCl	3.297	2371846	391746	7154	1.2
4	Clonidine HCl	3.215	2361748	391847	9917	1.2

5	Clonidine HCl	3.254	2371649	384622	9247	1.2
Mean			2378830			
Std.dev			14958			
%RSD			0.628797			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table: Results of repeatability for Chlorthalidone:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Chlorthalidone	5.441	198464	7291	6274	1.1
2	Chlorthalidone	5.442	193643	7219	6592	1.1
3	Chlorthalidone	5.409	196462	7194	6028	1.1
4	Chlorthalidone	5.520	194644	8174	6927	1.1
5	Chlorthalidone	5.424	198464	8653	5920	1.1
Mean			196335.4			
Std.dev			2190.191			
%RSD			1.115536			

Intermediate precision:

Day 1:

Table: Results of Intermediate precision for Clonidine HCl

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Clonidine HCl	3.211	2389572	395275	9375	1.2
2	Clonidine HCl	3.211	2391847	392175	9275	1.2
3	Clonidine HCl	3.210	2319472	312947	8265	1.2
4	Clonidine HCl	3.212	2306842	310585	6254	1.2
5	Clonidine HCl	3.211	2375972	310694	9028	1.2

6	Clonidine HCl	3.297	2396746	358373	8928	1.2
Mean			2363409			
Std. Dev.			39730.83			
% RSD			1.681082			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

Table: Results of precision for Chlorthalidone

S.No	**Peak Name**	**RT**	**Area (µV*sec)**	**Height (µV)**	**USP Plate count**	**USP Tailing**
1	Chlorthalidone	5.411	197284	7194	8264	1.2
2	Chlorthalidone	5.410	197849	7294	9174	1.2
3	Chlorthalidone	5.420	196572	7147	9164	1.2
4	Chlorthalidone	5.423	195028	7927	9733	1.2
5	Chlorthalidone	5.419	199474	8238	9194	1.2
6	Chlorthalidone	5.409	197482	7638	8973	1.2
Mean			197281.5			
Std. Dev.			1466.354			
% RSD			0.74328			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

Day 2:

Table: Results of Intermediate precision Day 2 for Clonidine HCl

S.No	**Peak Name**	**RT**	**Area (µV*sec)**	**Height (µV)**	**USP Plate Count**	**USP Tailing**
1	Clonidine HCl	3.211	2389562	391741	9264	1.2

2	Clonidine HCl	3.233	2381654	391047	9746	1.2
3	Clonidine HCl	3.244	2381946	391748	9816	1.2
4	Clonidine HCl	3.297	2391741	391746	9917	1.2
5	Clonidine HCl	3.297	2386452	381641	9742	1.2
6	Clonidine HCl	3.202	2374763	381645	9017	1.2
Mean			2384353			
Std. Dev.			6183.339			
% RSD			0.25933			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

Table: Results of Intermediate precision Day 2 for Chlorthalidone

S.No	**Peak Name**	**RT**	**Area (µV*sec)**	**Height (µV)**	**USP Plate Count**	**USP Tailing**
1	Chlorthalidone	5.411	197486	7582	6272	1.1
2	Chlorthalidone	5.410	197486	7184	6174	1.1
3	Chlorthalidone	5.420	196746	7456	5184	1.1
4	Chlorthalidone	5.405	195862	7814	6194	1.1
5	Chlorthalidone	5.409	196582	7194	6292	1.1
6	Chlorthalidone	5.463	198463	7745	6191	1.1
Mean			197104.2			
Std. Dev.			903.542			
% RSD			0.458408			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

6.3.4: ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

The accuracy results for Clonidine HCl

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1217218	112.5	112.4	99.6	99.3
100%	2397141	225	225	100	
150%	3514547	337.5	332.5	98.5	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

The accuracy results for Chlorthalidone

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	98598.67	22.5	22.4	99.9	99.6
100%	198359.7	45	45	100	
150%	291512.3	67.5	66.8	99	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Clonidine HCl:

Result:

=3.3×39762/10421

=12.5µg/ml

Chlorthalidone:

Result:

=3.3×5008/4365
=3.7µg/ml

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Clonidine HCl :

Result:

=10×39762/10421

=38.1µg/ml

Chlorthalidone:

Result:

=10×5008/4365
=11.4µg/ml

ROBUSTNESS

Table: Results for Robustness

Clonidine HCl

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/min	2391746	3.202	9028	1.2
Less Flow rate of 0.9mL/min	2371831	3.639	7381	1.2
More Flow rate of 1.1mL/min	2218319	2.859	9311	1.1
Less organic phase (about 5 % decrease in organic phase)	2294821	3.460	7462	1.2

More organic phase (about 5 % Increase in organic phase)	2394811	3.022	6817	1.1

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table: Results for Robustness

Chlorthalidone

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.1mL/min	194627	5.463	7398	1.1
Less Flow rate of 0.9mL/min	183738	6.250	6883	1.1
More Flow rate of 0.8mL/min	198373	4.863	9917	1.2
Less organic phase (about 5 % decrease in organic phase)	178471	6.196	8372	1.1
More organic phase (about 5 % Increase in organic phase)	189462	5.010	7716	1.2

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY AND CONCLUSION

Summary

Summary of Validation Parameters for Clonidine HCl and Chlorthalidone by Developed RP-HPLC Method

Parameters	Clonidine HCl	Chlorthalidone
Retention Time (min.)	5.463	3.202
Linearity (µg/ml)	15-75µg/ml	75-375µg/ml
Correlation Coefficient (r^2)	0.999	0.999
Slope	4365	10421
Y - intercept	454.8	48956
LOD (µg/ml)	3.7	12.5

LOQ (µg/ml)	11.4	38.1
Repeatability (% RSD) n=6	1.1	0.6
Intraday Precision (% RSD) n=6	0.7	1.6
Interday Precision (% RSD) n=6	0.4	0.2
Accuracy (%)	99.6	99.3

CONCLUSION

The developed HPLC method offers several advantages such as rapidity, usage of simple mobile phase and easy sample preparation steps. Further, improved sensitivity makes it specific and reliable for its intended use. Hence, this method can be applied for the analysis of pure drug and pharmaceutical dosage forms.

From the present study it can be concluded that the proposed method is simple, sensitive, precise, specific, accurate and reproducible. Results of validation parameters demonstrated that the analytical procedure is suitable for its intended purpose and meets the criteria defined in ICH Q2A/R1.

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