Research Article

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LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR ANALYSIS OF CLONIDINE IN PHARMACEUTICAL DOSAGE

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Abstract:

A new simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid chromatography assay has been developed and validated for the estimation of CLONIDINE in tablet formulation. The separation was achieved by using C-18 column (250x4.6mm, 5µm in particle size) at ambient temperature coupled with a guard column of silica in mobile phase Methanol : Acetonitrile : water with the pH value adjusted to 5.8 .The flow rate of was 1.5ml/min and the drug was detected using UV detector at the wavelength of 280nm and the run time was 6min. The retention time was within 2.6minutes. The percentage of RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per ICH guidelines. The proposed method was found to be accurate, repeatability and consistent. It was successfully applied for the analysis of the drug in marketed formulation and could be effectively used for the routine analysis of formulation containing the drug without any alteration in the chromatography conditions.

Key Words:

Clonidine, RP HPLC, UV Detection, 280 nm, Validation, C18 column.

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Introduction:

Clonidine is 2-imidazole derivative, it is a adrenergic agonist. It was discovered in the course of testing the drug for use as a topically applied nasal decongestant and used to treat blood pressure. Clonidine is a central acting antihypertensive drug which has been

approved for the Dutch market since 1968. Clonidine is an agonist of the α2-receptor and the imidazoline (I1) receptor in the central nervous system. Stimulation of these receptors results in several pharmacological effects, including a decrease of the blood pressure and heart rate. The innovator product Dixarit (Clonidine 0.025mg) is registered for migraine prophylaxis and menopausal flushing when estrogens are contraindicated or not tolerated and its registration is limited to treatment of adults and children of 12 year and older[1]. The innovator product Catapressan (Clonidine 0.15mg) is not available as tablet anymore. Generic products with Clonidine (0.025, 0.1 and 0.15 mg) have a wider indication than Dixarit and are also registered for hypertension and withdrawal reactions after cessation of opiates[2]. In clinical practice Clonidine is used as well for indications like attention deficit disorder (ADHD), insomnia due to ADHD, alcohol withdrawal symptoms and as adjuvant to opiates for analgesia[3].It relaxes and dilates blood vessels which results in lower blood pressure. Clonidine is used as withdraw associated symptoms of narcotics and benzodiazepines.

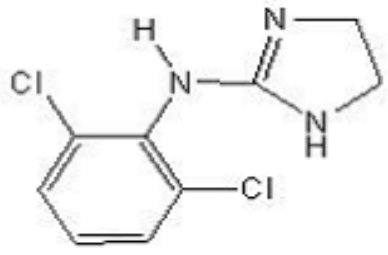


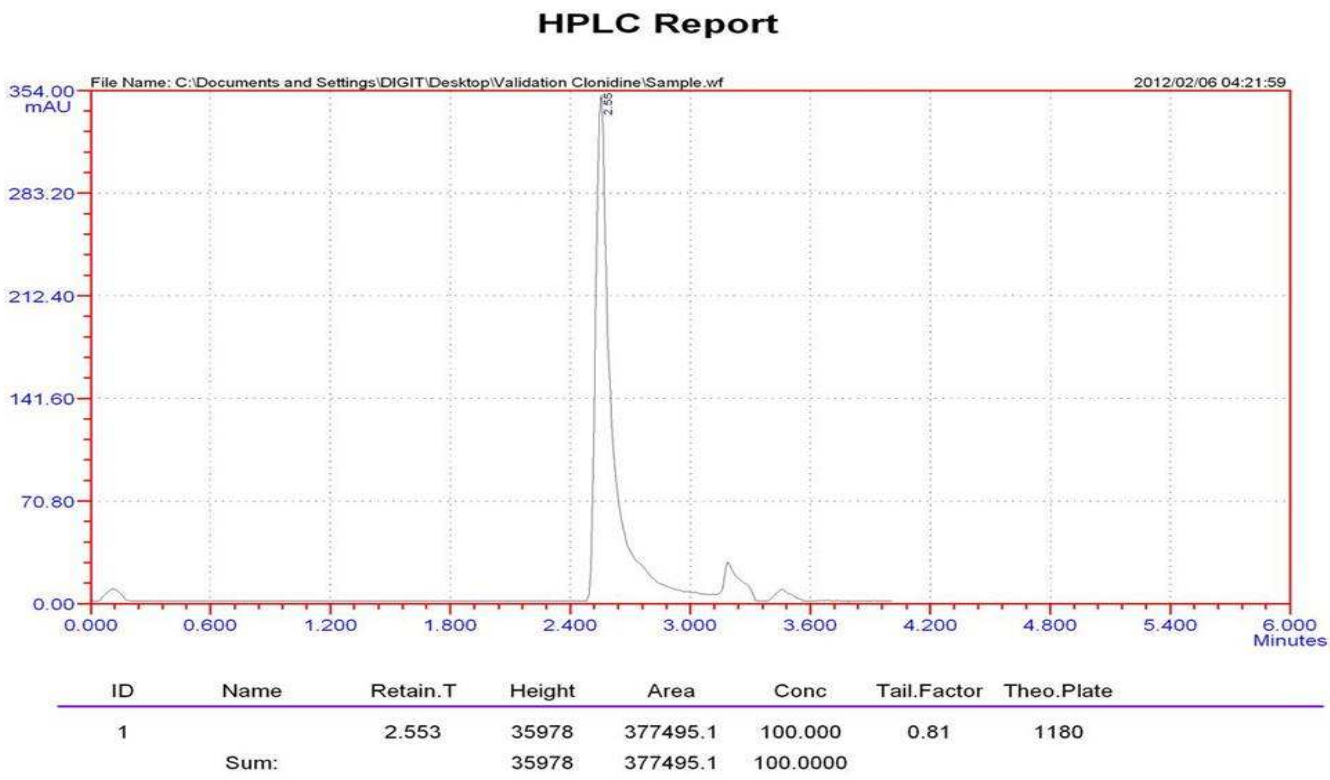
Figure 1 Structure of Clonidine

Methods and Materials

Working standard of CLONIDINE was obtained from well reputed research laboratories. HPLC grade Methanol acetonitrile water. (E.Merck, Mumbai, India)

S.N	Parameters	Result
1	Standard concentration	40µg/ml
2	Mobile phase	Methanol : Acetonitrile : water {80:10:10}
3	Wave length	280nm
4	P^H	5.8
5	Flo w rate	1.5ml/min.
6	Retention time	2.687 min.
7	Run time	5min.
8	Peak area	378627.8
9	Theoretical plates	4876.51
10	Pump pressure	4.4psi

Table 1: Chromatographic Conditions of Clonidine



Sample chromatogram of Clonidine in hplc

Different mobile phases were tried but satisfactory. The separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methanol : Acetonitrile : Water (80:10:10).The retention time of CLONIDINE was found to be 2.68min with pressure 4.4psi, which indicates a good base line. The system suitability and validation parameters are given in Table 4. The high percentage of recovery of CLONIDINE was found to be 100.44%indicating that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of CLONIDINE in tablet formulation. The result for CLONIDINE was comparable with a corresponding labelled amount (Table 6). The absence of additional peaks indicates no interference of the excipients.

Chemicals and reagents

The Tablets of combined dosage form were procured from the local market. Other reagents used like Acetonitrile which are of HPLC grade water were purchased from E.Merck, Mumbai, India

Analytical conditions

The development and validation of the assay was performed on A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column zodiac C18. 250×4.6mm, manual injector Rheodyne valve with 20µL

fixed loop, PEAK LC software was used. The mobile phase consisted of a Methanol:ACN:Water(80:10:10) Injections were carried out using a 20µl loop at room temperature and the flow rate was 1.5ml/min. Detection was performed at 280nm with 6min runtime.

Instrumental Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column zodiac C18. 250×4.6mm, Electronic balance-DENVER (SI234), a manual Rheodyne injector with a 20µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 SPECOPHOTOMETER was used to determine the wavelength of maximum absorbance.

Determination of wavelength of maximum absorbance

The standard solutions of clonidine were scanned in the range of 200 -400nm against mobile phase as a blank. CLONIDINE showed maximum absorbance at 280nm. So the wavelength selected for the determination of CLONIDINE was 280nm.

Preparation of Stock, working standard solutions

10 mg amount of CLONIDINE reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000ppm concentrated solution. From standard solution by the serial dilution we prepared required concentrations of 100 ppm..

Preparation of sample solution

A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of CLONIDINE was accurately weighted and quantitatively transferred into a 100ml volumetric flask. Approximately 50ml mobile phase were added and the solution was sonicated for 15min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 40µg/ml.

Method validation procedure

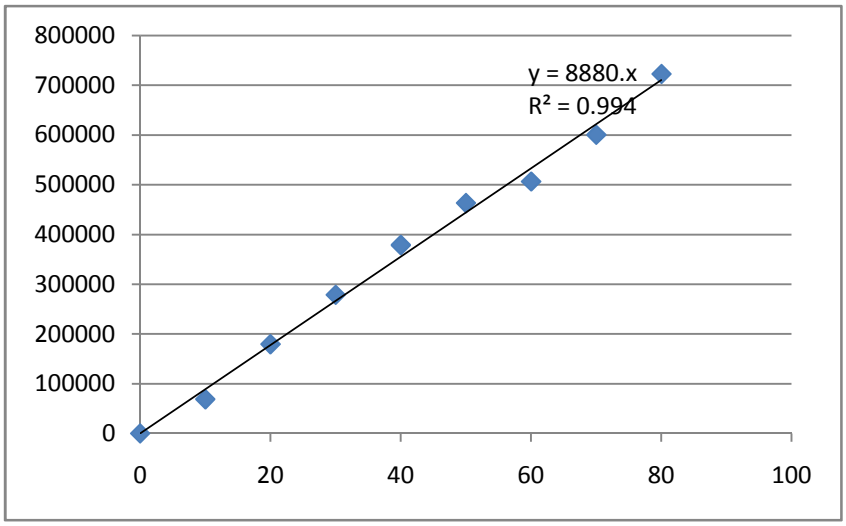
The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines for linearity, precision, system suitability, specificity, limit of detection and limit of quantification and robustness.

Range of linearity

Standard curves were constructed daily, for three consecutive days, using nine standard concentrations in a range of 10, 20, 30, 40, 50, 60, 70, 80, µg/ml for Clonidine. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = **8880** (r= 0.994)

Linearity

S.No	Conc. (PPM)	Area	
1	10µg/ml	68586.3	Slope:8880 Intercept:0.199166 CC: 0.994
2	20µg/ml	179275.7	
3	30µg/ml	278541.5	
4	40µg/ml	378627.8	
5	5oµg/ml	463067.3	
6	60µg/ml	506419.5	
7	70µg/ml	600509.9	
8	80µg/ml	722582.7	



Calibration curve of CLONIDINE

Precision

To study precision, six replicate standard solutions of CLONIDINE (100ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in

S.N	Concentrations	Area	RSD
1	40µg/ml	368739.1	
2	40µg/ml	360480.6	
3	40µg/ml	361078.5	
4	40µg/ml	369426.1	**1.6**
5	40µg/ml	356749.7	
6	40µg/ml	367368.9	

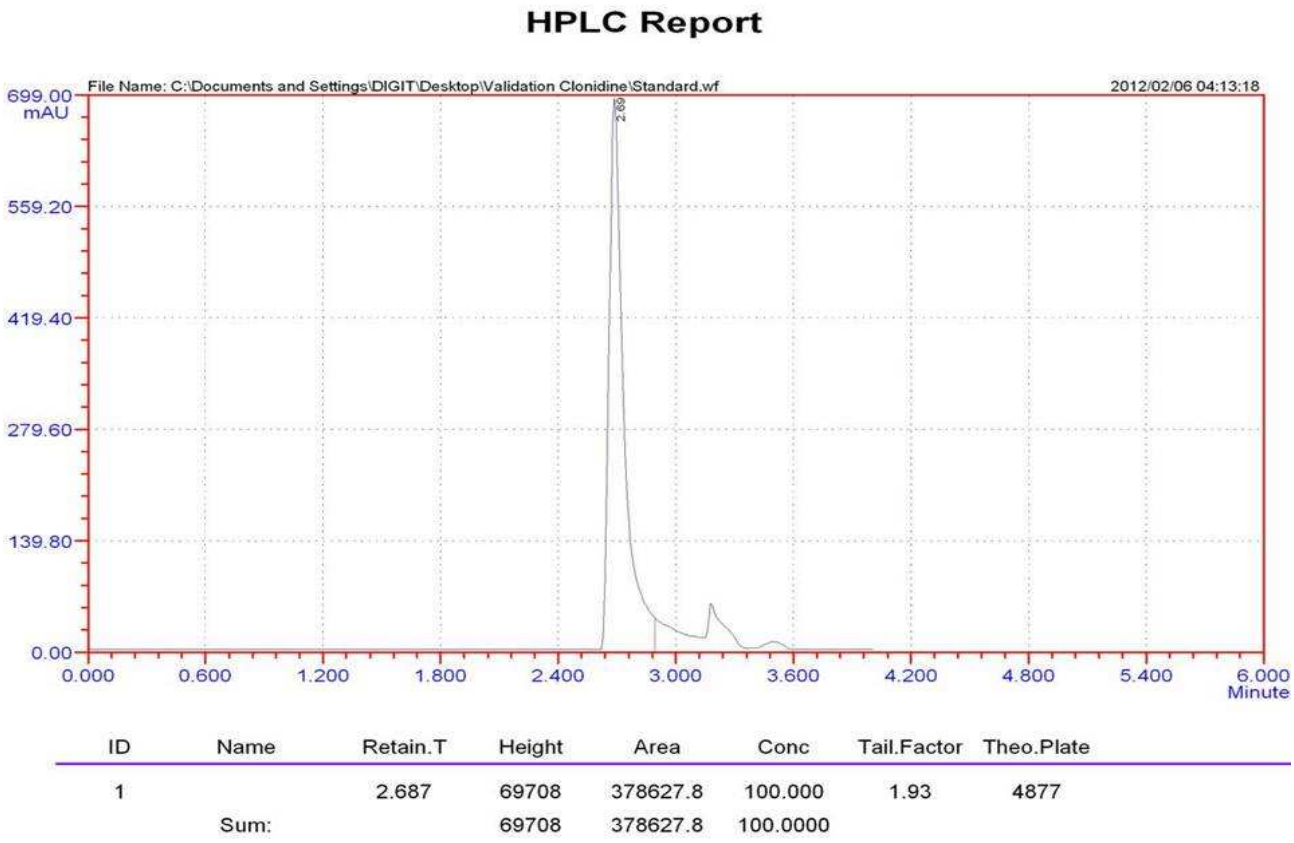
Table 3: Precision parameters of Clonidine

Ruggedness

S.N	Concentration	Area	RSD
1		408240.8	
2		408428.9	
3		409569.4	1.83
4	40µg/ml	408800.8	
5		396078.6	
6		392929.1	

System Suitability

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000 13. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.3



Standard chromatogram of Clonidine

Specificity

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing recipients with that of test results those obtained from standard drug.

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.0781ppm dilution Peak was not clearly observed, based on which 0.0781ppm is considered as Limit of Detection and Limit of Quantification is 0.25773ppm.

Limit of detection (LOD):

The Limit of Detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value.LOD can be calculated as:

LOD = 0.0781 ppm

Limit of detection:

S.N	Concentration	Retention Time (min.)	Area	Absorbance
1	0.0781 µg/ml	2.717	38320.8	Peak absorbed
2	0.0390625 µg/ml	…….	…….	No Peak

Table: 6

Limit of quantification (LOQ):

The Limit of Quantization (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy.

LOQ = 0.25773ppm

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above and the results are given in Table 7

Sample (40µg/ml)	Retention Time (min.)	Area	% Of recovery	% Of difference
Wave length 280nm Flow rate 1.5ml/min	2.68	378627.8	100	0.0
Wave length 278 nm	2.709	379769.4	100.3	0.3
Flow rate 1.3ml/min	3.042	377378.1	99.66	0.33
Mobile phase methanol:water:Acetonitrile 85:7.5:7:7.5	2.432	385024.6	101.6	1.6

Robustness parameters of Clonidine

Recovery

Recovery test was performed at 3 different concentrations i.e. 20ppm, 40ppm, 60ppm. Results are given in Table 8

Recovery	Concentration of sample	Drug recovery	% Drug recovery	Average % Recovery
50%	20 µg/ml	19.7 PPM	98.95	99.8%
100%	40 µg/ml	40.004 PPM	100.01	
150%	60 µg/ml	60.26	100.44	

Results and Discussion

Optimization of the chromatographic conditions

The Sample nature, its solubility and molecular weight confirms the correct selection of the stationary phase. The drug CLONIDINE being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. The elution of the compound from the column was influenced by polar mobile phase. The concentration of the acetonitrile and water were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained.

Tablet Formulation

Formulation	Concentration	Amount found	% Assay
NEXICLON Tablet	40ppm	39.881	99.7

Table: 9 Assay results of formulation

Conclusion

The proposed method for the assay of CLONIDINE in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for linearity, precision, system suitability, specificity, LOD & LOQ and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

Acknowledgement

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