A VALIDATED HPLC METHOD FOR THE ESTIMATION OF CYCLOBENZAPRINE HYDROCHLORIDE BY QUALITY BY DESIGN APPROACH IN BULK AND ITS TABLET DOSAGE FORM

Abstract:

Cyclobenzaprine hydrochloride is used to treat muscle spasms brought on by acute, uncomfortable musculoskeletal diseases. The primary objective of this work is to develop accurate, quick and precise HPLC method for Cyclobenzaprine hydrochloride. The method used is a Central Composite Design to get exact data for the procedure. Various parameters were used to validate and to develop method. The optimized model for the analysis of Cyclobenzaprine Hydrochloride was found to have an area 8.3239. This optimization was done on the C18 analytical column with mobile phase as methanol: 0.01 % Orthophosphoric acid (61:39 v/v) and a flow rate maintain was 0.9 ml/min with a detection wavelength of 224 nm. With the use of a correlation coefficient (r2=0.999), the linearity of Cyclobenzaprine hydrochloride in the 5–25 ug/ml range was determined. The accuracy values were discovered to fall between 99.86 and 100.71%. While the robustness was shown to be less than 0.06 for flow rate and for wavelength was 0.09 % RSD, also the intraday and interday precision were determined to be under 0.28 and 0.29 % RSD**.**

Result: The most effective approach for analysing Cyclobenzaprine hydrochloride is the one that has been presented.

Conclusion: The development and validation of the HPLC technique for Cyclobenzaprine hydrochloride were assessed.

Keywords: Cyclobenzaprine Hydrochloride, HPLC method, Validation, Central Composite Design, Quality by Design.

INTRODUCTION:

Muscle relaxants that operate centrally include Cyclobenzaprine hydrochloride [3-(5H-dibenzo [a,d] cyclohepten-5-ylidene]-N-N-dimethyl-1-propanamine hydrochloride (CB)], which is linked to tricyclic antidepressants..[1] It was first synthesised in 1961 and was made available for human use

from 1977. The molecular formula and molecular weight of Cyclobenzaprine hydrochloride is C20H21N and 275.4 g/mol respectively. Although the precise mechanism of action of this medication is still unclear, it works largely on the brain stem and decreases the somatic motor activity by inhibiting both alpha as well as gamma nerve fibres, which further decreases muscular spasm. To treat muscular spasm and acute musculoskeletal pain, Cyclobenzaprine hydrochloride is used as a short-term therapy for around 2-3 weeks, coupled with resting and some physical therapy. Its off-label indications include pain reduction and reduction in sleep disturbances in patients with fibromyalgia.[2]

Quality By Design (QBD) is a methodology that incorporates analytical, statistical, and threat methodologies into the design to guarantee the quality of Cyclobenzaprine hydrochloride. We chose the quantity of mobile phases and flow rates using the Central Composite design.[3]

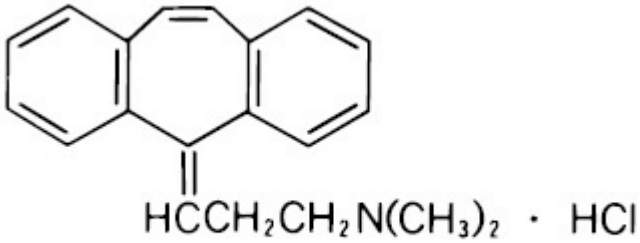
MATERIAL AND METHODS:

Material:

Chemicals:

Chart 1. Chemicals diagram

Cyclobenzaprine
Hydrochloride



The above diagram is referred from.[4]

Methods:

Design of Experiment:

Central Composite Design:

The Central Composite Design (CCD) useful experimental design in statistics for response surface approach. This design contains three distinct types of experimental runs, including:

It consists of three different types of runs of experiments, including:

1. A group of star points, sometimes referred to as axis points.

2. A group of centres that are frequently duplicated to increase the experiment's accuracy.

We employed variables like Mobile phase and Flow Rate in our investigation.

Using a factorial design gives researchers the ability to alter or include any variable when needed over the course of the experiment. In the Central Composite Factorial experimental design Mobile phase used is a Combination of Methanol and Water. Independent factors which were selected were time of retention, Area of Peak, Theoretical plates and Peak symmetry. C-18 column was chosen to separate Cyclobenzaprine HCl from other compounds. A typical run is provided by this design, but only for single mobile phase once at time.

Table no 1: Factors considered for the study by software used.

Sr. No	Composition of Mobile phase (Organic Phase)	Flow Rate	Retention Time	Theoretical Plate
1	62	0.9	3.145	7596
2	60	0.7	4.275	8305
3	62	0.7	4.339	10535
4	60	0.7	4.391	9160
5	62	0.7	4.307	9441
6	62	0.9	3.234	8856
7	60	0.9	3.783	8206
8	60	0.9	3.319	8458

Table no 2: Optimised Chromatographic Conditions

Sr. No	Amount of Methanol: 0.01 % OPA	Flow Rate	Retention Time	Symmetry	Theoretical Plates	Area
1	61:39	0.9	3.326	0.80	10555	8.3239

Mobile phase preparation:

61 ml of methanol and 39 ml of 0.01 % Orthophosphoric acid was adjusted i.e 61:39 v/v preparations. This solution was further filtered and then sonicated for 30 minutes.

Stock solution preparation of Cyclobenzaprine HCl:

The stock solution was made by mixing 10 ml of methanol with 5 mg of cyclobenzaprine hydrochloride. For a concentration of 500 g/ml, further dilutions were done.

Selection of Wavelength for detection:

Water was used to perform dilutions from the standard stock solutions, and after that, the spectrum of 200-400 nm was examined. The medication displayed its absorption at 224 nm in wavelength.

RESULT AND DISCUSSION:

Validation Method:

Validation method is a procedure where a number of assessments are designed in order to verify that a particular analytical method such as HPLC in our studies is suitable for the intended use or not.

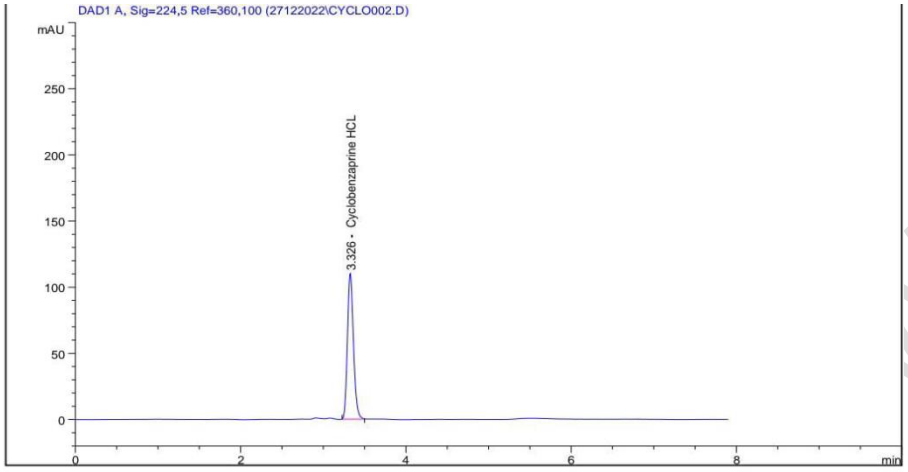
Precision, linearity, accuracy, and robustness were taken into consideration while determining if the HPLC process complied with the International Conference on Harmonization's (ICH) guideline Q2(R1) standard.

Range and Linearity: For Cyclobenzaprine hydrochloride, the range of the calibration curve's five levels was in the range of 5 to 25 ug/ml. Cyclobenzaprine HCL stock solution was diluted to five different known amounts. Concentrating on the (x-axis) and area (y-axis), the graph plot was used to determine the coefficient of correlation, slope, and y-intercept.

Table no 3: Linearity result

Sr.No	Concentrations (µg/ml)	Area of peak
1	5	286.21
2	10	556.19
3	15	831.51
4	20	1118.44
5	25	1410.44

Fig 1: Calibration Curve Graph of Cyclobenzaprine Hydrochloride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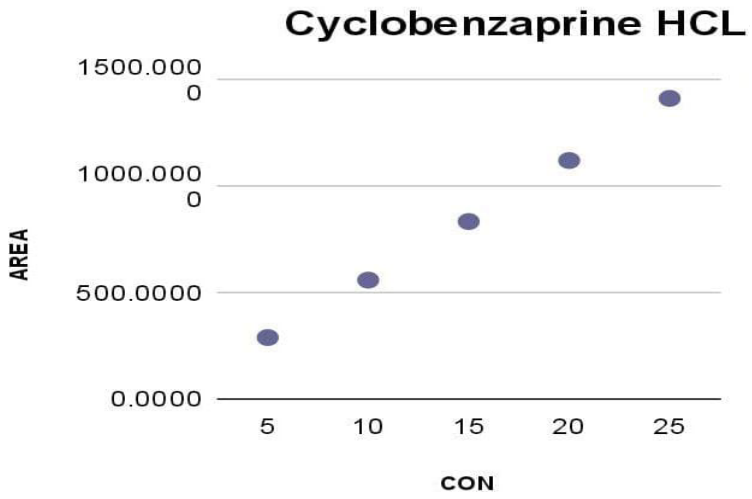
Cyclobenzaprine HCL
1500.000
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1000.000
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10
15
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25
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Table no 4: Characteristics Parameters for Presented HPLC method

Sr. No	Parameters	Results
1	Range of calibration (µg/ml)	5-25
2	Wavelength of Detection(nm)	224
3	Solvent system (CH_3OH: 0.01% OPA)	61:39
4	Regression equation (y*)	56.21x-2.65
5	Slope (b)	56.1
6	Intercept (a)	2.653
7	Correlation of coefficient(r2)	0.999
8	LOD (µg/ml)	0.09143
9	LOQ (µg/ml)	0.2770

System Suitability: The appropriateness of the system is crucial for method development. For 6 injections of 15 ug/ml Cyclobenzaprine hydrochloride, factors such retention duration, value of number of theoretical plates (N), area, asymmetry, and tailing factor (T) value were evaluated. The outcomes shown in the table below fall within the acceptable range.

Table no 5: Studies on Cyclobenzaprine HCl's system suitability using the HPLC method

Sr. No	Properties	Result
1	Retention Duration	3.326
2	Area	560.89

3	Symmetry	0.80
4	Theoretical Plates	10296
5	Tailing Factor	0.92

Precision: Precision was performed under two classes, Intra-Day and Inter-Day precision. Cyclobenzaprine HCL was injected six times to test the system's accuracy. Further the %RSD and average for these six determinations were determined. Additionally, in order to exhibit the method of Precision, 6 Samples from the similar Batch were separately examined and an assay was performed to determine the content. The reports of our results are displayed in the table below:

Table no 6: Intraday Precision at 224 nm

Concentration	RT	Peak Area Concentration
5	3.325	286.45
15	3.326	840.32
25	3.328	1408.45

Table no 7: Inter-day Precision at 224 nm

Concentration	RT	Peak Area Concentration
5	3.335	286.83
15	3.213	837.88
25	3.324	1407.29

Accuracy: Recovery test was conducted to verify the accuracy of the HPLC method by standard addition method. Evaluation of previously examined samples of cyclobenzaprine hydrochloride were done by spiking them with 80%, 100% and 120% of cyclobenzaprine hydrochloride standard and then their combination was analysed. Also, the percentage of RSD and the Standard deviation recovery were examined and reported. Results are mentioned in the given table.

Table no 8: Accuracy of Cyclobenzaprine HCL at 224 nm

Sr.No	Concentration	Found concentration	Recovery percentage
1.	80	4 ug/ml	100.71
2.	100	5 ug/ml	100.79
3.	120	6 ug/ml	99.86

Robustness: Robustness refers to a method's capacity to endure little but deliberate modifications, which foretells the method's reliability. In order to see whether the method is robust or not, the conditions of the experiment were changed at three different levels which are as follows:

1. Change in Mobile phase
2. Change in flow rate
3. Change in detection wavelength.[5–12]

The following table lists the results:

Table no 9: Robustness Studies

Concentration	Flow rate-0.8	Wavelength-224
15	748.207	792.15
15	749.25	793.21
15	747.14	792.13

15	750.124	794.7
15	748.102	791.04
15	749.38	793.91

Tablet assay:

Weight of 20 tablets were taken and then mean value was calculated. Further the sample Preparation (20 ug/ml) was made and drug content per tablet was determined by performing assay.

Conclusion:

The QBD technique is used in the present experimental investigation to show the creation and validation of a quick and easy HPLC technique to identify cyclobenzaprine hydrochloride in its pure state. This developed experiment is cheaper as compared to its counterparts reported in other studies. This method is highly reliable due to its precision, accuracy and sensitivity. It can further be reproduced to be applied for the purpose of quality control.

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