Asian Journal of Chemical Sciences

Volume 14, Issue 1, Page 50-57, 2024; Article no.AJOCS.112039
ISSN: 2456-7795

A Validated HPLC Method using C18 Analytical Column (Agilent) for the Estimation of Cyclobenzaprine Hydrochloride by Quality by Design Approach in Bulk and Its Tablet Dosage Form

Deepak D. Pokharkar [a*], Raghavdeep Sharma [a], Shreelekha N. Desai [a] and Lekha Dhake [a]

[a] NCRD's Sterling Institute of Pharmacy, Plot No. 93/93A, Sector - 19, Nerul (E), Seawoods, Navi Mumbai, Maharashtra 400706, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOCS/2024/v14i1285

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/112039

Original Research Article

Received: 27/11/2023
Accepted: 02/02/2024
Published: 06/02/2024

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

**Corresponding author: E-mail: deepak.pokharkar@ncrdsip.com;*

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(Agilent) 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. The most effective approach for analysing Cyclobenzaprine hydrochloride is the one that has been presented. 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.

1. 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 [2,3]. 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 [4,5-7].

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 [8,9-11].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals

Cyclobenzaprine Hydrochloride

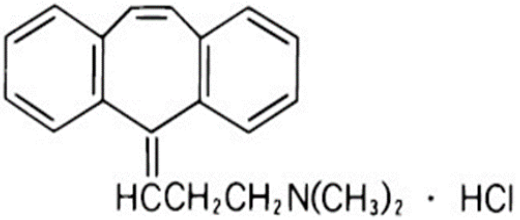


Chart 1. Chemicals diagram

The above diagram is referred from [12].

2.2 Methods

2.2.1 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.

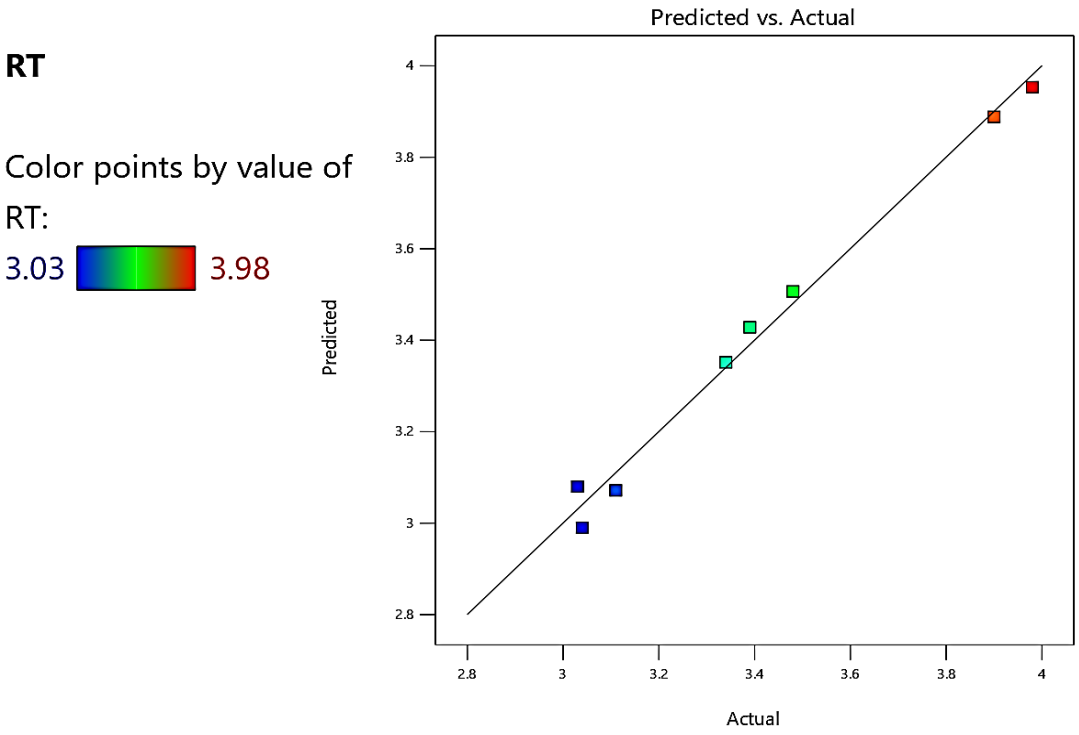
The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Table 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 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



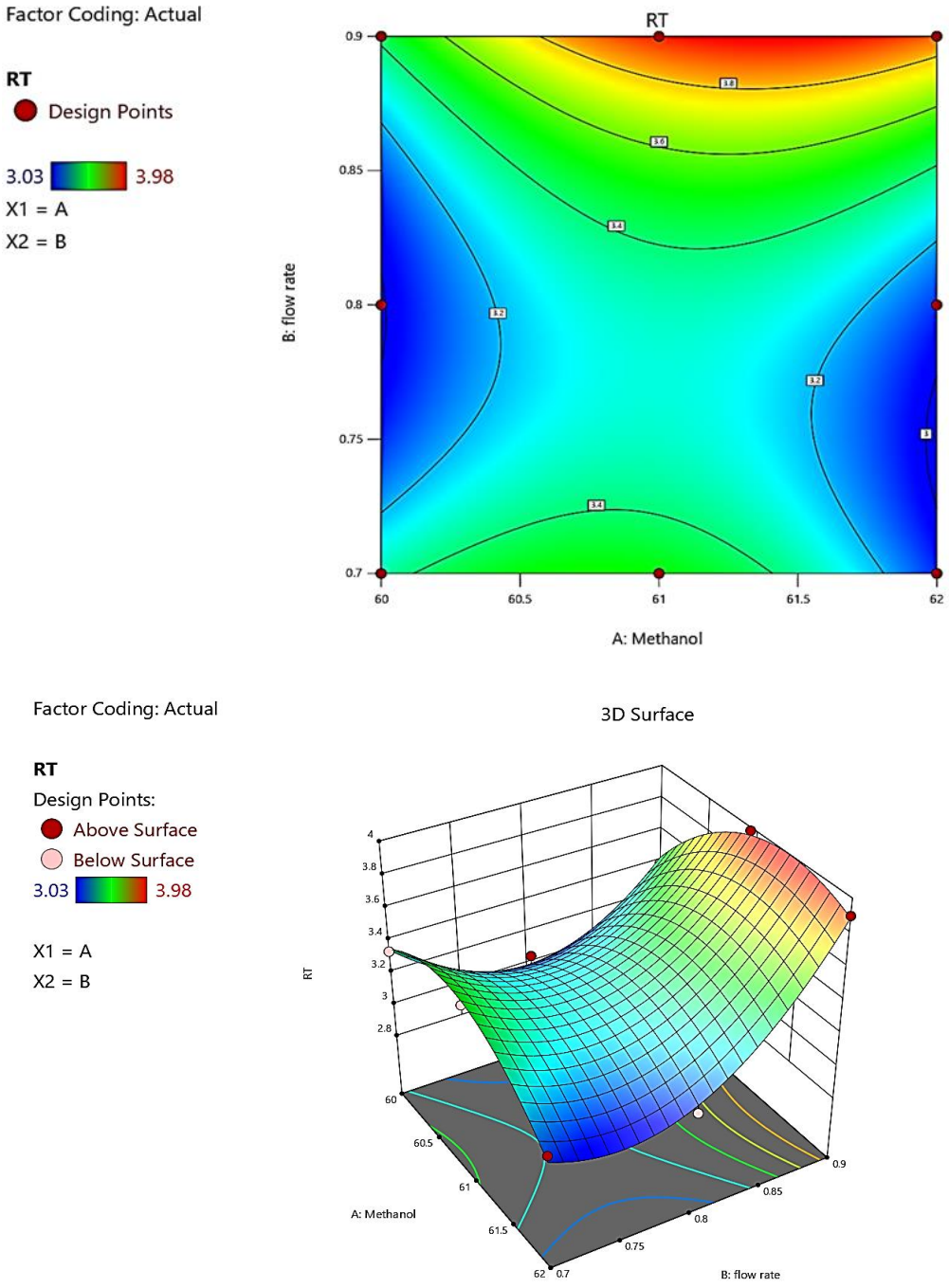


Fig. 1. Coded and actual factors with 3D design

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.

3. RESULTS 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 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

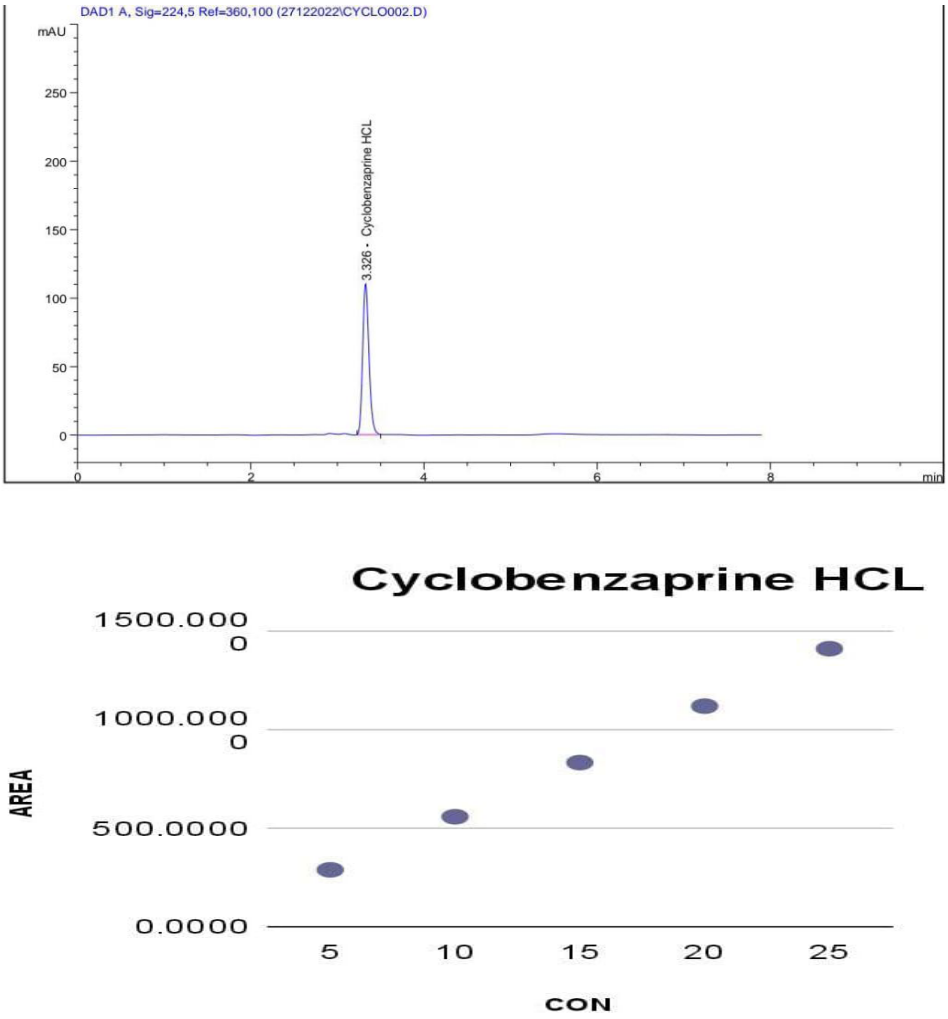


Fig. 2. Calibration curve graph of cyclobenzaprine hydrochloride

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.

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 6.

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 8.

Table 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

Table 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

Table 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 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

Table 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

Table 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

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 [13–20].

The Table 9 lists the results

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.

4. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. NK R, TA M, RM F, AA M. Development and validation of spectrophotometric and spectrofluorimetric methods for the determination of cyclobenzaprine HCl. Pharm Anal Acta. 2017;09(01).
2. Hany W. Darwish, Ibrahim A Naguib, Ibrahim A Darwish. Five modified classical least squares based models for stability indicating analysis of cyclobenzaprine HCl with its major degradation products: A comparative study.
3. Yasmini T Parshuramkar, Milind J Umekar, Krishna R Gupta. Development and validation of dissolution test method for determination of cyclobenzaprine hydrochloride from its formulation using HPLC.
4. Available:https://go.drugbank.com/drugs/DB00924
5. annasaheb S Gaikwad, Monika N Madibone, Jeevan R Rajguru, Prashant B Jadhav. Development and validation of RP-HPLC and UV spectrophotometric absorptivity method for simultaneous estimation of cyclobenzaprine hydrochloride and aceclofenac in pharmaceutical dosage form.
6. Cyclobenzaprine hydrochloride-cyclobenzaprine hydrochloride tablet, film coated amneal pharmaceuticals LLC.
7. US Pharmacopeia, 25th edn, US pharmacopeial convention, Inc., Rockville, MD. 2007;1846.
8. Babar SA, Padwal SL. QBD approach to analytical method development and its validation for estimation of lenvatinib in bulk and pharmaceutical formulation. International Journal of Applied Pharmaceutics. 2021 Sep 1;13(5):183–8.
9. Sensitive kinetic spectrophotometric determination of cyclobenzaprine HCl in pure form and pharmaceutical formulations, Mohammed A. Kassem chemistry department, faculty of applied sciences, umm al-qura university, Makkah, Saudi Arabia; chemistry department, faculty of science, benha university, Benha13518, Egypt.
10. Ramadan NK, Mohamed TA, Fouad RM, Moustafa AA. Development and validation of spectrophotometric and spectrofluorimetric methods for the determination of cyclobenzaprine HCl.

11. Annnasaheb S Gaikwad, M Madibone. Development and validation of RP-HPLC and UV-spectrophotometric absorptivity method for simultaneous estimation of cyclobenzaprine hydrochloride and aceclofenac in pharmaceutical dosage form.
12. Gaikwad AS, Madibone MN, Rajguru JR, Jadhav B. Development and validation of RP-HPLC and UV-spectrophotometric absorptivity method for simultaneous estimation of cyclobenzaprine hydro-chloride and aceclofenac in pharma-ceutical dosage form. IJSDR2004056 International Journal of Scientific Development and Research [Internet]; 2020.
13. Santosh M, Waghamare A, Sumithra M. Full factorial experimental design for development and validation of Rp-Hplc method for estimation of apixaban in bulk and pharmaceutical formulations. Journal of Pharmaceutical Negative Results. 2022;13.
14. Available:https://www.semanticscholar.org/paper/Analytical-Method-Development-and-Validation-for-of-Waghmare-Kale/4bf27a19305669adcf72002d4e2d9b2c94fe5541#:~:text=A%20simple%2C%20sensitive%2C%20rapid%20and%20reproducible%20UV%20method,Expert%20software%20by%20applying%203%20level%20factorial%20design.
15. Journal E, Giri SS, Pukale AK, Waghamare SA Snehal et al. Spectroscopic method development and validation for estimation of apixaban in bulk and pharmaceutical formulation by QbD approach [Internet]. Available:www.ejbps.com
16. Waghmare SA, Kashid AM. Reverse phase-high performance liquid chromatography method development and validation for estimation of efavirenz by quality by design approach. Journal of Drug Delivery and Therapeutics. 2019 Feb 15;9(1-s):319–30.
17. Rajkotwala AS, Sirajuddin SS, Dedania ZR, Dedania RR, Vijendraswamy SM, Rajkotwala India AS. “QbD approach to analytical method development and validation of piracetam by hplc.” World journal of pharmacy and pharmaceutical sciences SJIF impact factor 6 [Internet]. 2016;5(5):1771–84. Available:www.wjpps.com
18. Available:https://www.semanticscholar.org/paper/Quality-by-Design-(QbD)-Based-Development-and-of-a-Garg-Sharma/7c4cec0bf34ed99760eea7a46d344689ecd4d727
19. Mandlik SK, Agarwal PP, Dandgavhal HP. Implementation of quality by design (Qbd) approach in formulation and development of ritonavir pellets using extrusion spheronization method. International Journal of Applied Pharmaceutics. 2020 Jan 1;12(1):139–46.
20. Available:https://www.semanticscholar.org/paper/ANALYTICAL-QUALITY-BY-DESIGN-APPROACH-IN-RP-HPLC-OF-Ch-A./a2f1f6e6bfef0f2f0a4d847c5de5c0702c14da44

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/112039