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Cleaning Method Development and Validation of Brimonidine Tartrate Residue from Brimonidine Tartrate API

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Abstract

Objectives: To Develop the Precise, Linear and Accurate Cleaning method Validation for the Brimonidine Tartrate from Brimonide tartrate Active Pharmaceutical Ingredient as per International Conference on Harmonization guidelines by using High Performance Liquid Chromatography.

Material and Methods: In this method uses a reverse phase C_{18} column make as inert sustain AQ C_{18} column (250 × 4.6 mm, 5µ), mobile phase-a as 0.1% ortho phosphoric acid and mobile phase-b as acetonitrile : methanol (50:50) v/v , flow rate as 1.2 ml/min, and detection of wavelength AS 248 nm, column temperature 40°C, injection volume 10µL and autosampler temperature 25°C by using Photo Diode Array Detector.

Results: In this method Brimonidine tartrate peak was eluted at 3.9 minutes. Brimonidine tartrate linear range was 0.5 µg/mL to7.5 µg/mL. In System suitability the % Relative standard deviation observed as 0.5. In swab and Rinse samples recovery was found between 97% to 103%. The limit of Quantification and limit of Detection range is found to be 0.5µg/mL and 0.15 µg/mL respectively.

Conclusion: An accurate, linear and precise Reverse phase High Performance Liquid Chromatography method was developed and validated as per International Conference on Harmonization guidelines. Hence this cleaning method validation is used for routine analysis of the residue samples in various pharmaceutical manufacturing areas.

Keywords: *Reverse phase High performance liquid chromatography; Method Development; Cleaning Method validation; Brimonidine tartrate*

Abbreviations

RP-HPLC: Reverse Phase High Performance Liquid Chromatography, ICH: International Conference on Harmonization, S/N : Signal to Noise, LOQ: Limit of Quantitation, LOD: Limit of Detection, RSD: Relative Standard Deviation.

Introduction

Cleaning method validation is used to establish the procedure to avoid contamination residues of the active pharmaceutical ingredients of the product manufactured in the reactors. Which are also predetermined levels to ensure the quality of the next product. Cleaning method validation is used to demonstrate the procedure for Cleaning of different drug substances and drug products. This method is used to avoid the contamination for another drug substances and drug products. Cleaning method validation is also one part of the good manufacturing practices [1].

In cleaning method validation two types of techniques used in sample collection.

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1. Swab Technique

2. Rinse Technique

Brimonidine tartrate is used to treat pressure in the eye. The pressure in the eyes which leads to vision loss and sight and which also causes the blindness also [2].

Cleaning method is which decrease the chance of contamination. In case of manufacturing of drug substances (Active pharmaceutical ingredients) the contamination is very big problem. The contamination which leads to increasing of impurities and by products and degradants which causes the increasing the potent nature and which gives the side effects. So in order to solve this problem cleaning method validation is developed. This is the requirement for many manufacturing companies for production of active pharmaceutical ingredients [3].

Experiment

Molecular Formula : $C_{15}H_{16}BrN_5O_6$

Molecular Weight : 442.22g/mol

Solubility : 0.154 mg/mL in water

Pka (Strongest Basic) : 8.32

This data was collected from the Brimonidine Tratrate Web Chem. By Based on the Structure, Solubility and Pka values method was developed. By based on the Structure Brimonidine tartrate is the polar molecule. So Reverse phase column shall be selected for to develop the method and Acidic mobile phase 0.1% Ortho Phosphoric acid and methanol is used because the molecule is basic [4].

Materials and Method

Orthophosphoric acid, methanol and acetonitrile were of HPLC grade and were procured from Merck India. HPLC grade water for preparation of mobile phase was purchased from Reputed Laboratory. Brimonidine Tartrate API was provided by Biocon company, Hyderabad, India.

Cleaning method development and validation was performed using Agilent 1260 high performance liquid chromatograph with Quarter nary pump, auto sampler, photodiode detector and a column compartment. Data was processed by using open lab software. Inert sustain AQ C_{18} column was used.

Chromatographic conditions

A reverse phase HPLC Cleaning method was developed by System Suitability parameter by injecting Blank one injection and Standard injected as 6 replicates and calculated the theoretical plates , Tailing factor and % Relative standard deviation found to be with in the acceptance criteria. Brimonidine Tartrate Peak was eluted at 3.9 minutes and no Blank Interference was observed and signal to Noise Ratio of Brimonidine Tartrate was more than 10. By using this method Brimonidine Tartrate was validated.

Cleaning method validation

The cleaning method validation parameters which are performed on the basis of the ICH guidelines. The method validation performed parameters like System Suitability, Precision, LOQ and LOD Determination, Linearity, Accuracy, Range, Solution Stability and Robustness [5].

System suitability

System suitability is used to determine the mobile phase, column and chromatographic conditions should be suitable for the Analyte. Inject Blank (one replicate) and Standard Solution (6 replicates) and calculated the % Relative standard deviation for Area of the Analyte and Theoretical plates and Taling factor. The limits for % Relative standard deviation for area of Brimonidine tartrate is not more than 10, Theoretical plates is not less than 2000 and Tailing factor is not more than 2.0 respectively.

Specificity

In specificity parameter Blank Interference is checked by after the system suitability injected blank and no interference was observed at the Brimonidine tartrate Retention time.

Precision

Method Precision (Repeatability): In method precision inject the 6 swab sample preparations as per chromatographic conditions and check the assay of the Analyte.

The % Assay value should be 80 to 120%.

Intermediate precision (Ruggedness): Intermediate Precision is performed by different analyst and different column and different HPLC system. In Intermediate precision inject the 6 swab sample preparations as per chromatographic conditions and check the assay of the Analyte.

The % Assay value should be 80 to 120%.

The cumulative % RSD for assay of 12 samples (Method precision and Intermediate precision)

Should be not more than 20%.

LOD and LOQ Establishment

LOQ=(10 x STEYX of Analyte)/Slope of the Analyte

LOD=(3 x STEYX of Analyte)/Slope of the Analyte

Injected the Linearity levels (25%, 50%, 80%, 100%, 120%, 150% and 200%) and calculated the Slope and STEYX of the analyte to determine the LOQ and LOD Concentration and performed the LOQ Precision

Linearity

Injected the Linearity levels (LOQ, 50%, 80%, 100%, 120% and 150%) and calculated the Slope and Intercept and Correlation coefficient (R) and Regression Coefficient (R2) and % Y-Intercept. The correlation coefficient and Regression coefficient value is not less than 0.990 and % Y- intercept not more than 5.

Accuracy (recovery) parameter

In Accuracy parameter prepare the levels to LOQ, 50%, 100%, 150% and 200% and calculated the recoveries. The recovery values should be with in 70 to 130% for LOQ Level and 80 to 120 for remaining levels.

Solution stability

The solution stability parameter which denotes the how many hours the solution is stable.

Robustness parameter

In Robustness Parameter Change in flow and column temperature performed and check the system the suitability parameters which are complies with the system suitability acceptance criteria [6-14].

Results and Discussion

By Based on the Structure, Solubility and Pka values method was developed. By based on the Structure Brimonidine tartrate is the polar molecule. So Reverse phase column shall be selected for to develop the method and Acidic mobile phase 0.1% Ortho Phosphoric acid and methanol is used because the molecule is basic in nature. Pka value of Brimonidine tartrate is 8.32. The system suitability results (TABLE 1), Specificity results (TABLE 2) , Method precision results (TABLE 3), Intermediate precision results (TABLE 4), LOD and LOQ establishment results (TABLE 5), LOQ Precision results (TABLE 6), Linearity results (TABLE 7), Accuracy Results (TABLE 8), Solution stability results (TABLE 9) and Robustness results (TABLE 10) were with complies with the acceptance criteria. Hence this is validated by using ICH Q_2(R) guidelines. By this method we can conclude that there is no contamination is observed for manufacturing of the any of the Active Pharmaceutical ingredients (FIGS. 1-3).

Chromatograms

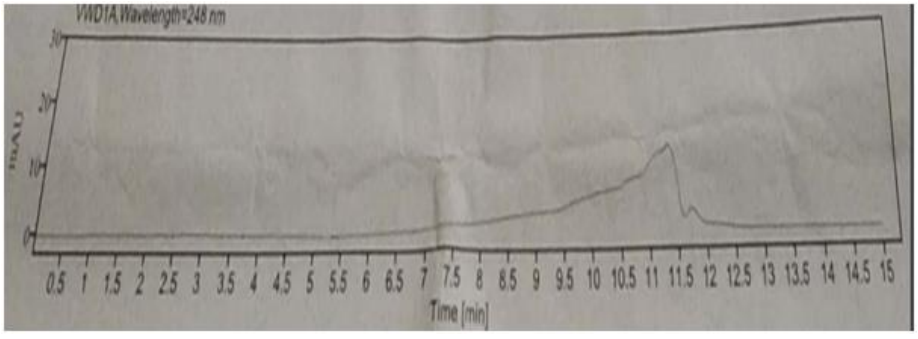


FIG.1. **Blank**

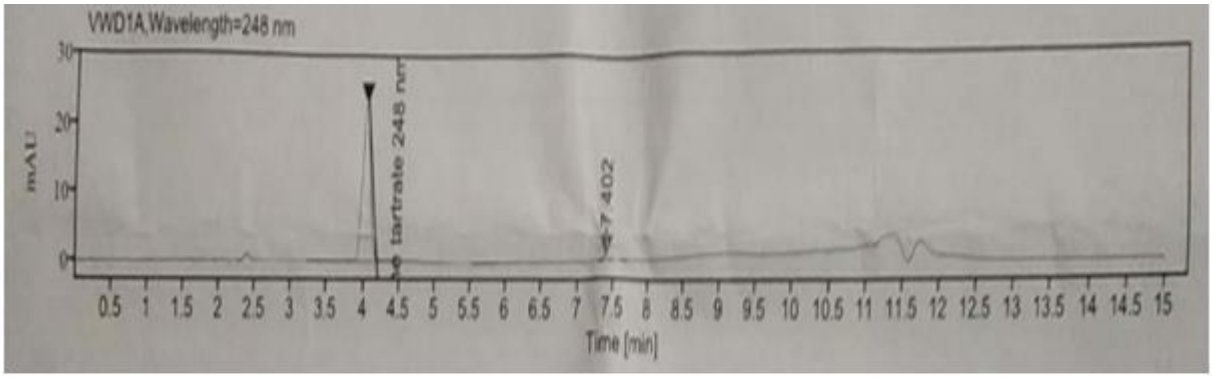


FIG.2. **Standard Solution**

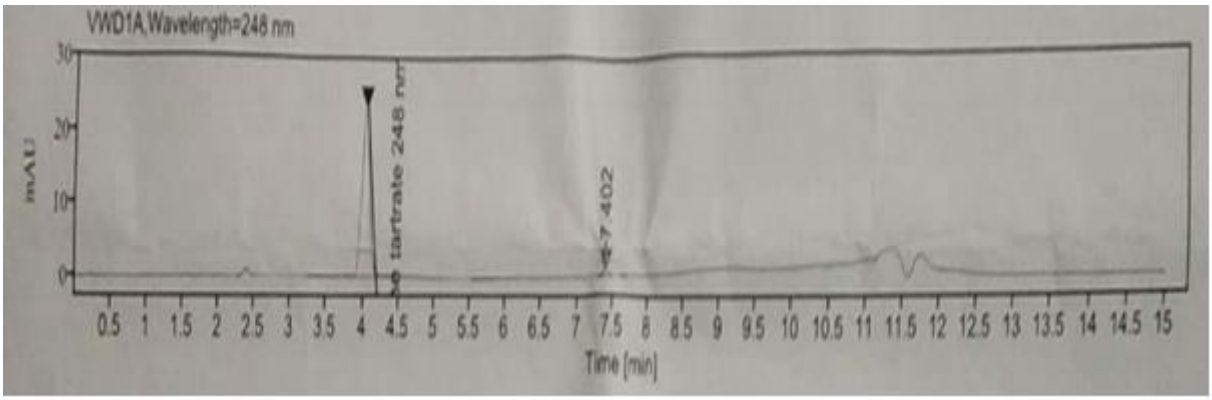


FIG.3. **Swab Sample Solution**

TABLE 1. **System suitability.**

System suitability parameter	Observed	Limit
Theoretical Plate of Brimonidine tartrate	15089	NLT 2000
Tailing Factor of Brimonidine tartrate	1	NMT 2.0
% RSD for Six Replicate of the of Brimonidine tartrate Standard	0.8	NMT 10

TABLE 2. **Specificity.**

Name of the Sample	**Retention time**
Brimonidine tartrate Standard	3.8 minutes
Blank	No interference

TABLE 3. **Method precision results for swab sample.**

S.NO	**Method precision Results**
1	98.3
2	97.6
3	98.7
4	98.9
5	97.8
6	97.5
Average for 6 Samples	98.1
Standard Deviation for 6 Samples	0.58878
% RSD for 6 Samples	0.6

TABLE 4. **Intermediate precision results for swab sample.**

S.NO	Intermediate precision Results
1	97.2
2	97.5
3	97.6
4	98.3
5	97.6
6	98.8
Average for 6 Samples	97.8
Standard Deviation for 6 Samples	0.59554
% RSD for 6 Samples	0.6

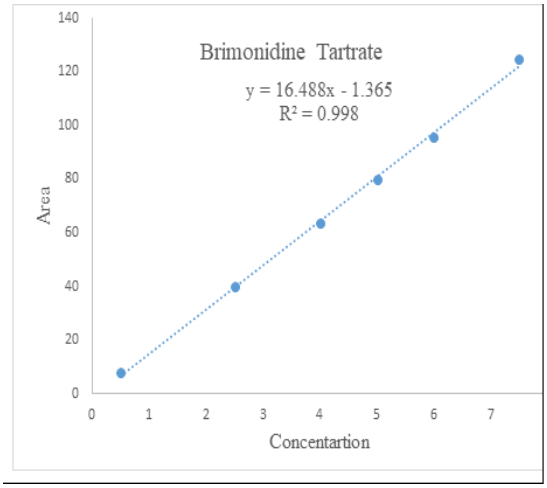
TABLE 5. **Lod and loq establishment table.**

Name of the Sample	Concentration
LOQ	0.5 ppm
LOD	0.17 ppm

TABLE 6. **Loq precision.**

S.NO	LOQ precision Area
1	7.925
2	7.856
3	7.875
4	7.83
5	7.755
6	8.059
Average	7.883
Standard Deviation	0.10269
% RSD	1.3

TABLE 7. **Linearity.**



Level	ppm	Area
LOQ	0.5	8.025
50%	2.5	40.05
80%	4	63.569
100%	5	80.025
120%	6	95.685
150%	7.5	124.895
Slope		16.488
Intercept		-1.365
Correlataion (R)		0.999
Regression (R2)		0.998
% Y-Intercept		-1.7

TABLE 8. **Accuracy.**

Level	% Recovery for Swab sample
LOQ-1	97.3
LOQ-2	98.4
LOQ-3	98.5
50%-1	98.9
50%-2	98.9
50%-3	98.7
100%-1	99.2
100%-2	99.6
100%-3	99.5
150%-1	99.8
150%-2	99.6
150%-3	99.8

TABLE 9. **Solution stability.**

Brimonidine Standard		**Swab Sample**	
Hours	**% Difference**	**Hour**	**% Difference**
6 hours	0.2	6 hours	0.3
12 hours	0.5	12 hours	0.4
24 hours	0.7	24 hours	0.6
36 hours	0.8	36 hours	0.7

TABLE 10. **Robustness.**

Condition	Retention time (mins)	% RSD for Area of analyte
As such condition	3.592	0.5
Flow minus (1.0 mL)	4.125	0.4
Flow plus (1.4 mL)	2.928	0.6
Column Temperature (35°C)	3.958	0.5
Column Temperature (45°C)	3.028	0.8

Conclusion

From the above results the Brimonidine tartrate is Linearly and Accurately précised. The production manufactured equipment's are cleaned by using this diluent. And there is no possibility to contamination of the drug substances.

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Conflict of Interest

The authors declare no conflict of interest.

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