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Assay Method Development And Validation of Carprofen Active Pharmaceutical Ingredient By Reverse Phase HPLC

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Abstract

A simple, specific, linear, sensitive, precise, efficient Reverse phase HPLC method was developed and validated for the Assay determination of Carprofen Active Pharmaceutical ingredient (API). The proposed method involves use of Inertsil C8; 10 cm x 4.6mm, 3µ column and mobile phase comprising of mixture of Water: Acetonitrile: Methanol: Glacial acetic acid (40: 40: 20: 0.2 v/v). The column temperature is maintained at 25ºC and flow rate at 1 ml/minute. Detector used is a UV (Ultra violet- Visible) detector at wavelength of 240 nm. The retention time of active ingredient is about 5 minutes. The method was validated for specificity, system suitability, linearity, precision and robustness. Method is specific as no interference was observed at Retention time of Principle peak due to Carprofen. The linear regression analysis data for the calibration plots showed a linear relationship over the concentration range of 50 ppm-250 ppm for active ingredient. Percentage RSD of active ingredient was found to below 2%. Statistical analysis showed that the method is repeatable and selective for the Assay determination of Carprofen Active Pharmaceutical ingredient (API.)

Keywords *– Carprofen, Reverse Phase HPLC, Validation, Assay*

1. INTRODUCTION

Carprofen is a nonsteroidal anti-inflammatory drug (NSAID). The chemical name of Carprofen is 2-(6-chloro-9H-carbazol-2-yl)propanoic acid. Molecular weight is 273.72 g·mol^{-1}. It is an anti-inflammatory drug In addition to anti-inflammatory actions, non-steroidal anti-inflammatory drugs have analgesic, antipyretic, and platelet-inhibitory actions. They act by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase, which converts arachidonic acid to cyclic endoperoxides, precursors of prostaglandins.[1]

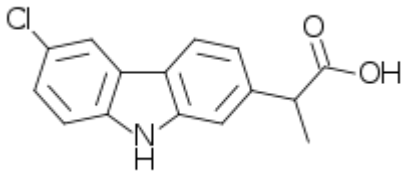

Fig.1 : Chemical Structure of Carprofen

Evaluation of available literature reveals that Literature survey reveals methods have been reported for assay determination of Carprofen drug substance as well as Carprofen containing finished drug product. However, there was no method available with short run time for Assay evaluation[2-6]. So an attempt was made to develop and validate a simple, rapid reverse-phase high performance liquid chromatographic method with short run time for the estimation of Carprofen in active ingredient.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Carprofen Sample and standard were gifted by commercial source. Glacial acetic acid (S.D fine), Acetonitrile (Rankem chem), Methanol (S.D fine), water (Milli -Q) were obtained from Merck chemicals. Distilled water was prepared using a Milli-Q system (Millipore). Nylon syringe filters (0.45 µm) were from Millipore.

2.2 Equipment

Chromatographic separation was achieved using HPLC System (Vanquish DUO series) containing PDA detector. The output signal was monitored and processed using Chemstation Software®. The analytical balance used was from Sartorius, Model-BSA224SCW.

2.3 Selection of UV Wavelength

Structure evaluation of Active ingredient confirmed presence of multiple number of chromophores e.g. Unsaturated double bonds as part of Benzene ring as well as presence of Carbonyl group. Based on these structural features we decided to adopt UV technique for detection purpose.

Further literature survey of available data in Public domain confirms reported UV max to be at about 240 nm. Hence, we decided to adopt the same as wavelength of detection for Assay determination of this Active ingredient.

2.4 HPLC Analytical Conditions

The proposed method involves use of Inertsil C8; 10 cm x 4.6mm, 3 µcolumn and mobile phase comprising of mixture of Water: Acetonitrile: Methanol: Glacial acetic acid (40: 40: 20: 0.2 v/v). The column temperature is maintained at 25ºC and flow rate at 1 ml/minute. Detector used is a UV (Ultra violet- Visible) detector at wavelength of detection for Assay determination is 240 nm. The retention time of active ingredient is about 5 minutes. Sample and standard preparation was done in a Mobile phase.

2.5 Preparation of standard solution

Weighed accurately about 100 mg of Carprofen working standard and into 100 ml volumetric flask. 25 ml of diluent was added, sonicated for 2 minutes, cool and dilute up to the mark with diluent. Further 1.5 ml of this solution was diluted to 10.0 ml with diluent.

2.6 Preparation of Sample solution

Weighed accurately about 100 mg of Carprofen working sample and into 10 ml volumetric flask. 25 ml of diluent was added, sonicated for 2 minutes, cool and dilute up to the mark with diluent. Further 1.5 ml of this solution was diluted to 10.0 ml with diluent.

2.7 Method validation

The developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, Validation of Analytical Procedures: Q2(R1)[7], for the parameters like Specificity, system suitability, linearity and range, precision (repeatability), and robustness.

2.8 System suitability

System suitability testing is essential component of all the analytical techniques used in Pharmaceutical industry, and they are based on principle that equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. These tests need to be established for each individual analysis to confirm validity of testing.

System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. The system suitability test performed using the standard solution and results were recorded to find the adequate percentage relative standard deviation for area, retention time, Tailing factor and theoretical plates.

2.9 Specificity

No blank interference should observe at retention time of peak due to Carprofen.

2.10 Precision

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

Method precision was evaluated by injecting six different sample preparation.

The assay of these samples was determined. Precision of the method was evaluated by calculating the % RSD.

2.11 Linearity

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration (amount)of analyte in the sample. The linearity of detector response was determined by preparing a series of solution of the working standards of active ingredient over the range of 50% to 150% of targeted concentration. These solutions were injected into the chromatographic system and response area was recorded.

2.12 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

A study was performed to determine the effect of variation in the temperature. Standard solution prepared as per the test method and was injected into the HPLC system at 25°C temperature.

3. RESULTS AND DISCUSSION

The UV max of Carprofen as reported in literature is 240 nm, so for quantification HPLC with UV detector was employed. To begin with development mobile phase comprising of water and Acetonitrile in the ratio of 50:50 v/v and zorbax SB C18 15cm*4.6mm*5 was used. Column oven temperature was 25ºC and flow rate of 1 ml was kept and 10 µl of 150 ppm of Carprofen in Acetonitrile was injected. It was observed that peak eluted at about 5 minutes and had tailing. As zorbax SB C18 column showed tailing. Inertsil C18 column with 25cm x 4.6mm x 5µ was used, still peak shape was not improved. Then the diluent was changed to Acetonitrile: Water 50:50 v/v still tailing was observed. Since Carprofen showed tailing 0.1% TEA was introduced in Mobile phase and pH was adjusted to 2 with Ortho phosphoric acid. It was observed that peak tailing was reduced but the peak shape was broad and Retention time of peak was about 8 minutes. To reduce the peak broadening mixture of Acetonitrile and methanol was employed and sample was injected. Peak broadening was reduced and retention was about 5 minutes. As it is an Assay method so to shorter the run time it was decided to employ fused core column. So Inertsil C8; 10 cm x 4.6mm, 3 µ column was used. It was observed that peak shape was good. Further optimization of chromatographic parameters was done to improve peak shape and reduce run time.

The finalized chromatographic conditions are shown in Table-1.

System suitability parameters proved that the proposed method suits for the Assay determination of estimation of Carprofen. Chromatogram for assay determination of Carprofen was found satisfactory on Inertsil C8; 10 cm x 4.6mm, 3 µ column. Drug peak was found symmetrical as observed from Tailing factor of the proposed method was satisfactory.

Representative chromatograms are shown in Fig.2 and 3.

System suitability parameters were given in Table-2. UV detection was set at 240.

The data of precision were given in Table-3 and 4. The percentage RSD value was less than 2%. The data for similarity factor was provided in Table -5.

Sensitivity of the method was good and also linearity was observed over a wide concentration range of 50 ppm to 250 ppm for Carprofen. The correlation coefficients for Carprofen was found to be within the limits for r^2=0.9999.

The linearity data were given in Table-6 and Fig.4.

Results of Robustness study are provided in Table no. 7.

So the developed method was said to be validated. By performing deliberate variation in temperature, it was observed that there were no marked changes obtained in the chromatograms, which demonstrated that the method developed is robust.

Table 1: Optimized chromatographic conditions for proposed HPLC method for Carprofen

Parameter	Chromatographic condition
Instrument	Thermofisher, Vanquish Duo
Column	Inertsil C8; 10 cm x 4.6mm, 3 µ column
Detector (wavelength)	Thermo UV detector (240 nm)
Mobile phase	Water: Acetonitrile: Methanol: Glacial acetic acid
Diluent	Mobile phase
Flow rate	1 ml/minute
Column Temperature2	25ºC
Injection volume	10 µl
Retention time of peak due to	About 5 minutes
Mode of Chromatography	Isocratic

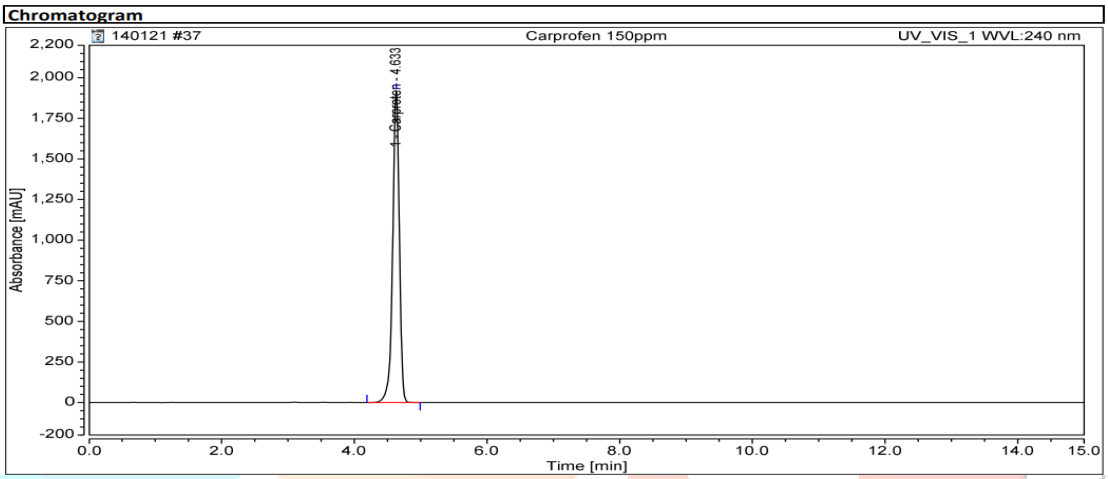

Fig. 2 : HPLC Chromatogram of Standard solution of Carprofen

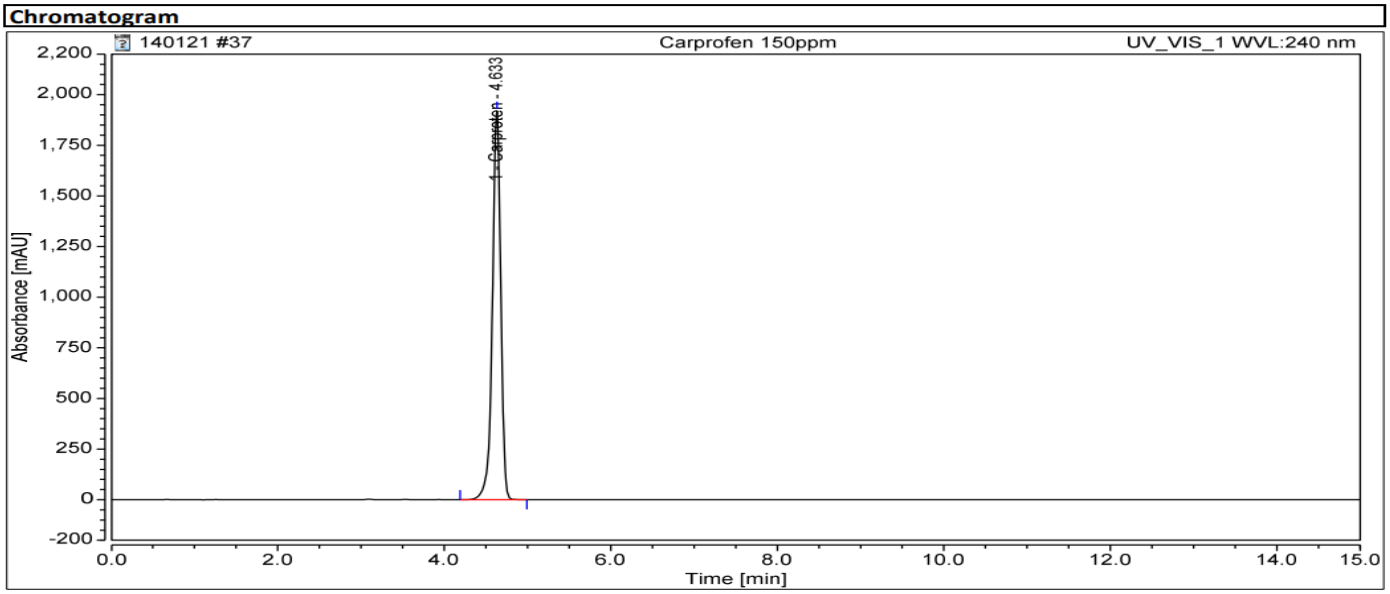

Fig. 3 : HPLC Chromatogram of Sample solution of Carprofen

Table 2: Results of System suitability

System suitability parameter	Result	Acceptance criteria
Similarity Factor	1.00	Between 0.98 & 1.02
% RSD of area response (For Five replicates of standard	0.06	NMT 2.0%
% RSD of Retention Time (For five replicates of standard	0.07	NMT 1.0%
Symmetry factor for first replicate of standard Preparation 1	1.9	Between 0.8 to 2.0
Column Efficiency for first replicate of standard	10762	NLT 2000
Symmetry factor for standard Preparation 2	1.9	Between 0.8 to 2.0
Column Efficiency for standard Preparation 2	10765	NLT 2000

Table 3: Results of System precision

Replicate no. of Standard solution	Retention time	Area
1	4.617	13625.08
2	4.617	13632.46
3	4.617	13636.61
4	4.625	13636.75
5	4.617	13640.11
6	4.617	13651.54
Mean	4.618	13637.09
Standard Deviation	0.003	8.76
% Relative Standard deviation	0.07	0.06
Acceptance criteria:	**% RSD for retention time should not be 2 %**	**% RSD for area response should not be 2 %**

Table 4: Results of Method Precision

Sample No.	Assay in %(on anhydrous basis)
1	99.6
2	99.9
3	99.6
4	99.5
5	99.5
6	99.4
Mean	99.6

Table 5: Results of Similarity Factor

Standard Preparation	Weight	Retention time	Peak Area Response
Mean of Peak area response of 6 replicates of System precision	100.45	----------	13637.09
Standard Preparation B	100.26	4.618	13611.29
Similarity Factor			1.0

Acceptance criteria: Similarity factor should be between 0.98 tom 1.02.

Table6: Results of linearity data

Linearity Level	Concentration (in ppm)	Mean Peak Area Response
L1	50	74.75
L2	100	149.39
L3	150	225.05
L4	200	308.03
L5	250	384.59

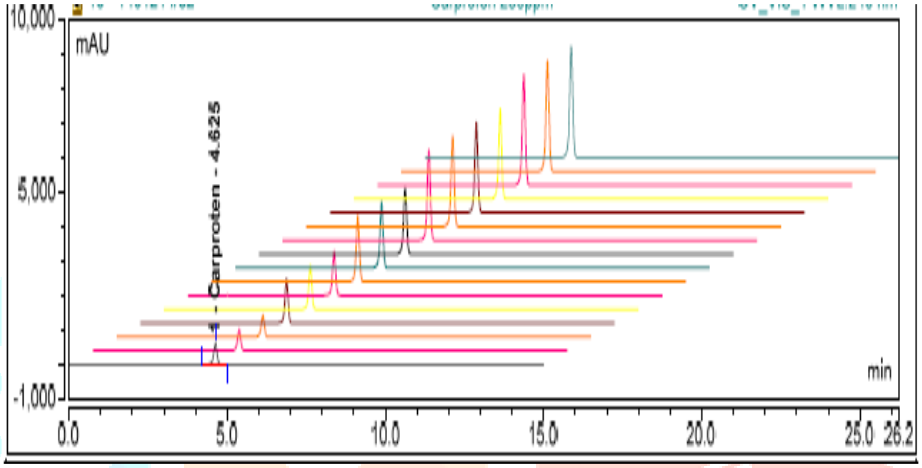

Fig. 4 Linearity chromatogram of Carprofen

Table 7: Results of Linearity and Range study

Observation for Carprofen	Results	Acceptance criteria
Correlation coefficient (r)	0.999	NLT 0.99
%Y-intercept	-2.3	NMT ± 3.0

Table 8: Results of solution stability study

Observation for Standard solution injected at varying temperature:

Parameter	Retention time of Carprofen Peak	Peak area response of Carprofen Peak
ROBUSTNESS for temperature changed to 35^0 C	4.51	229.36

4. CONCLUSION

The Reverse Phase HPLC method developed for Assay estimation of Carprofen is simple, specific, linear, sensitive, precise and efficient and is suitable for its intended purpose.

The method developed has shorter run time, thus ensuring optimum utilization of the HPLC system. Further the proposed method is on HPLC with shorter run time obtained generally with advance and costly techniques. Also shorter run time ensures lesser consumption of solvents in turn reducing further cost per analysis and also generating lesser solvent waste. The shorter run time enables analysis of multiple batches in short duration thus enhancing the out-put of batch analysis.

The method was validated as per ICH guidelines, showing satisfactory data for all the method validation parameters tested. Hence, the proposed method can be employed for assessing the Assay determination of Carprofen.

5. ACKNOWLEDGEMENT

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