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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF CARPROFEN IN BULK AND IN DOSAGE FORM

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ABSTRACT

A simple, sensitive and accurate stability indicating UV-spectrophotometric method has been developed for estimation of carprofen from bulk and pharmaceutical formulation. Method applied is area under curve (AUC) in which area under curve was integrated in the wavelength range of 250.5-280 nm. The λmax of carprofen in methanol:Acetonitrile (30:70) was found to be 261.5 nm. The drug follows linearity in the concentration range 1-5 µg/ml with correlation coefficient value 0.999. The proposed method was applied to pharmaceutical formulation and % amount of drug estimated 99.92% was found in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 3,4 and 5 µg/ml. The % recovery was found to be in the range 96%-99.25%. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % R.S.D. value less than 0.3 indicate that the method is precise. Ruggedness of the proposed method was studied with the help of two analysts. The above method was a rapid and cost-effective quality-control tool for routine analysis of carprofen in bulk and in pharmaceutical dosage form.

KEYWORDS

Carprofen, Stability Indication and Photostability of Carprofen.

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INTRODUCTION

Carprofen (Figure No.1) is one of the non-steroidal anti-inflammatory drug which is used in veterinarians for supportive treatment of various conditions. It used for treatment of pain and inflammation from arthritis in geriatric dogs, joint pain, osteoarthritis, hip dysplasia and other forms of joint deterioration. Carprofen reduces inflammation by inhibition of COX-2 and other source of inflammatory prostaglandins.

Carprofen (*RS*)-2-(6-Chloro-9*H*-carbazol-2-yl) propanoicacid derivate, was the first COX-2 selective drug approved for use in dogs. It is available in oral and injectable forms. The COX-2 selectivity of Carprofen renders the oral form effective for long- and short term pain management. The primary difference in pharmacokinetics between the oral and injectable forms is their peak plasma concentration after drug administration a single subcutaneous injection of Carprofen results in a lower peak plasma concentration than the oral administration of the same amount. Like other NSAIDs, Carprofen is highly protein bound in the blood, and it undergoes hepatic metabolism[1-3].

The mechanism of action of this compound is likely attributed to the inhibition of cyclooxygenase (COX) activity. There are two different COX enzymes that have been described in mammals COX-1 and COX-2. Compounds with activity against COX-1 enzymes were believed to affect the synthesis of prostaglandins important to normal gastrointestinal and renal function, while inhibition COX-2 enzymes were solely associated with altering anti-inflammatory activity. The constitutive cyclooxygenase, COX-1, synthesizes prostaglandins necessary for normal gastrointestinal and renal function. The inducible cyclooxygenase, COX-2, generates prostaglandins involved in inflammation[4-6].

Phototoxicity, photosensitivity disorders and hemolytic adverse effect induced by Carprofen (CPF) have been widely reported. The Photostability of CPF must be seriously considered with respect to its safety and efficacy in formulations. As the stability studies are important part of preformulation in dosage form development, establishing a stability indicating assay method suitable for potential critical factors has become an urgent task.

MATERIALS AND METHODS[7,8]

Equipment and Reagents

UV-Spectrophotometry was performed by using a Double beam UV - Visible spectrophotometer (Agilent technologies Carry 60) with 10mm matched quartz cells of capacity 1 ml was used. The bulk drug Carprofen was obtained as a gift sample form Jinan Jinda Pharmaceutical Chemistry Co. Ltd, China. Methanol and Acetonitrile (AR grade) were obtained from the research laboratory.

Preparation of solvent Methanol Acetonitrile solution

Measure 30ml of methanol and 70ml of acetonitrile separately. Transfer these solvents in 100ml volumetric flask and mix well.

Preparation of standard stock solution

10 mg Carprofen was accurately weighed and dissolved in small quantity of above prepared solvent mixture transferred to a 100 ml volumetric flask sonicate it for 5 min, finally volume was made up to the mark with the same mixture to get 100µg/ml stock solution.

Estimation of λmax

The solution of 6 µg/ml concentration was prepared by diluting standard drug solution with methanol: acetonitrile and scanned between 200-400 nm wavelength regions. The wavelength of maximum absorption was found to be at 261.5 nm.

Construction of calibration curve

Calibration curve was plotted against concentration and absorbance, regression equation was computed. The results tabulated in the Table No.1 and Figure No.2.

Conformity of λmax

Overlay spectra of standard drug were taken by scanning the solutions of different concentrations between the wavelength region 200-400nm and the wavelength of maximum absorption was found to be at 261.5nm (Figure No.3).

Estimation of carprofen in pharmaceutical formulation[9]

20 tablets were weighed and powdered. The amounts of tablet powder equivalent to 10 mg of Carprofen was weighed accurately and transferred to 10ml volumetric flask containing 5-6 ml of (Me: ACN), the solution was shaken at 50-60 rpm for 15 min volume was made with Me:ACN. The solution was then filtered through Whatmann filter paper # 41. 1ml filtrate was diluted to 10ml to get the solution of 100µg/ml concentration. Then remove 0.5ml from 100µg/ml solution diluted with Me:ACN up to 10ml and absorbance was taken at 261.5 nm in quantitative mode of the instrument and

concentration of solution is obtained by using calibration curve. The result obtained were analyzed statically and represented in Table No.2.

Experimental[10]

Method A: Calibration curve of Carprofen

Standard carprofen stock solution (0.1, 0.2, 0.3, 0.4, 0.5ml**)** was transferred in to different 10 ml volumetric flask. The volume was made up with (Me:ACN). Absorption of solution was measured at 262nm against Me:ACN as blank. The procedure was repeated for six times. The linearity was found to be between 1-5µg/ml and is shown as like molar absorptivity, regression equation, standard deviation on slope and intersept, correlation are also determined and is reported in Table No.1 and Figure No.4.

Mehtod B: Area under Curve of Carprofen[11]

Standard carprofen stock solution (0.2, 0.3, 0.4, 0.5ml**)** was transferred in to different 10ml volumetric flask. The volume was made up with (methnol:ACN). Absorption of solution was measured at 262nm against Me:ACN as blank. The procedure was repeated for six times. The linearity was found to be between 1-5µg/ml and is shown in like molar absorptivity, regression equation, standard deviation on slope and intercept, correlation are also determined and is reported in Table No.3 and Figure No.5.

Validation of the Method[12]

The method was validated with respect to linearity, accuracy and precision for the Carprofen as per ICH guidelines, as summarized here.

Linearity

The methods were validated according to International Conference on Harmonization Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte[13]. The linearity was evaluated by analyzing different concentrations of standard solution of Carprofen, but linearity was found to be between 1-5 µg/ml concentrations. The calibration graphs were obtained by plotting the absorbance versus the concentration data (Figure No.4).

Precision

Six replicate analysis of tablets by the proposed method were done. The results of the precision study indicate that the method is reliable. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts at different days in same laboratory. Results are shown in Table No.4.

Variation in result on the same day (intraday), variation in result between days (inter day) were analyzed. Intraday precision was determined by analyzed the three different concentration of the drug for three times in the days. Inter day precision was determined by the analyzing the three different concentration of the drug daily for the three days. Results are shown in Table No.5.

Accuracy (recovery test)

The accuracy of the method is the closeness of the measured value to the true value for the sample. Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts to tablet. The recovery was performed by preparing concentration of 80%, 100% and 120% of the label claim of the tablet. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The % recovery of the added pure drug was calculated as % recovery = [(*Ct*–*Cs*)/*Ca*] x 100, where *Ct* is the total drug concentration measured after standard addition; *Cs*, drug concentration in the formulation sample; *Ca*, drug concentration added to formulation. The results were as shown in Table No.6.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of Carprofen were determined from the data obtained from the linearity studies, by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. For each of the six replicate determinations, y intercept was calculated and the standard deviation of the y intercept was computed. From these values, the parameters LOD and LOQ were calculated on the basis of response

and slope of the regression equation. Result shown in Table No.7.

$$LOD=3.3\delta /S$$

$$LOQ=10\ \delta /S$$

Where, δ= standard deviation of residuals from the curve;

S=slope of the curve.

RESULTS AND DISCUSSION

The method A (absorption maxima) and method B (AUC) was developed and validated as per ICH guideline. Carprofen has the absorbance maxima at 261.5 nm (method A) (Figure No.2) and in the AUC spectrum method areas were measured between 250.5 nm to 280 nm. (Method B) (Figure No.3). The regression data for the calibration plots showed good linear relationship in the concentration range of 1-5 µg/ml and given in Table No.4. Recovery studies were carried out by adding the pure drug to the previously analyzed tablet powder sample and shown in Table No.6. The percentage recovery value indicates no interference from excipients used in formulation.

Table No.1: Data of calibration curve method A

S.No	Concentration (µg/ml)	Absorbance Mean ±S.D (n=5)	RSD
1	1	0.1169 ± 0.0009	0.22
2	2	0.2273 ± 0.0003	0.25
3	3	0.3237 ± 0.0003	0.24
4	4	0.4252 ± 0.0004	0.21
5	5	0.5252 ± 0.0001	0.22

Table No.2: Estimation of Carprofen from its Pharmaceutical Formulation

S.No	Formulation	Label claim	Amount found ±SD	RSD	% Recover
1	Tablet	50mg	49.96 ± 0.0215	0.0054	99.92
2	Tablet	50mg	49.66 ± 0.0205	0.0041	99.32

Table No.3: Data of Area under Curve method B

S.No	Concentration (µg/ml)	AUC (n=3)
1	2	8.79
2	3	9.22
3	4	9.8
4	5	10.18

Table No.4: Optical characteristics and precision data

S.No	Parameter	Method A	Method B
1	Absorption maxima	262nm	250-280
2	Molar Absorptivity	0.2948×10^{8}	-
3	Beers law limit (mcg/ml)	1-8	1-8
4	Regression equation	Abs = 0.099 conc + 0.014	Abs = 0.475 conc+ 7.835
5	Slope	0.099	0.475
6	Intercept	0.014	7.835
7	Correlation coefficient	0.996	0.994

Table No.5: Intra Day and Inter Day Precision Data of calibration curve

S.No	Concentration(µg/ml)	Intra Day(n=3)	RSD	Inter Day(n=3)	RSD
1	1	0.1161	0.22	0.1147	0.35
2	2	0.2200	0.25	0.2195	0.11
3	3	0.3162	0.24	0.3134	0.11
4	4	0.4126	0.12	0.4106	0.03
5	5	0.5184	0.21	0.5104	0.03

Table No.6: Accuracy Data (Recovery test)

S.No	Formulation	Amount added µg/ml	Amount of recovery µg/ml	% Recovery	Mean Recovery
1	Tablet (method A)	3 4 5	2.84 3.92 4.98	98.68 98.68 99.60	98.98
2	Tablet (method B)	3 4 5	2.88 3.97 3.49	96 99.25 98.20	97.81

Table No.7: LOD and LOQ data

S.No	Data	Method A	Method B
1	LOD	0.1232	0.4029
2	LOQ	0.3733	1.221

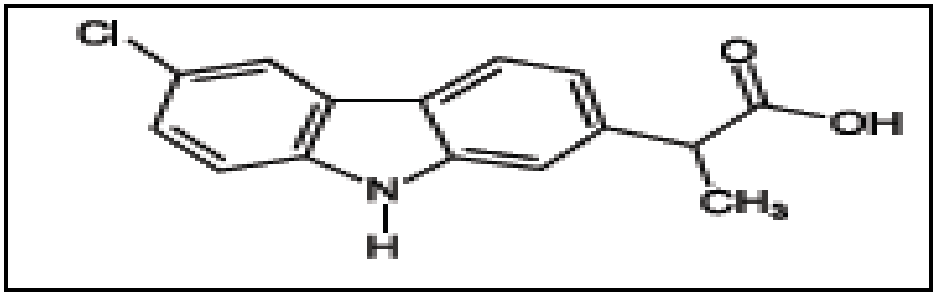

Figure No.1: Chemical structure of Carprofen

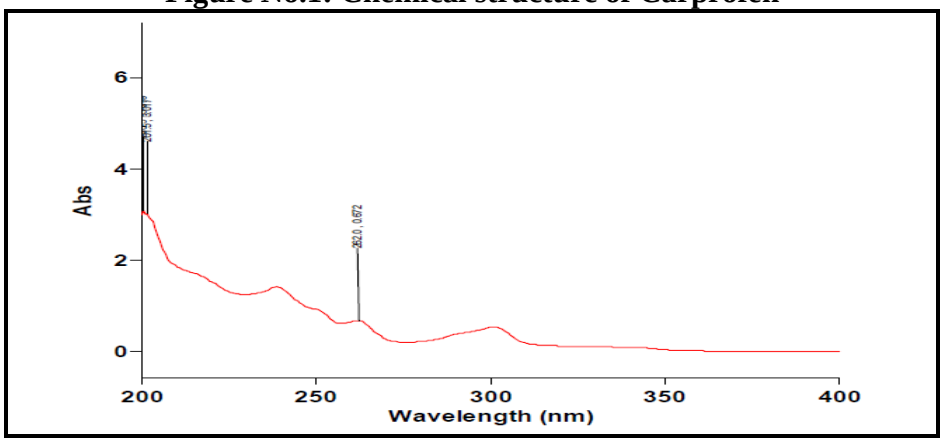

Figure No.2: Absorption Maxima for Carprofen

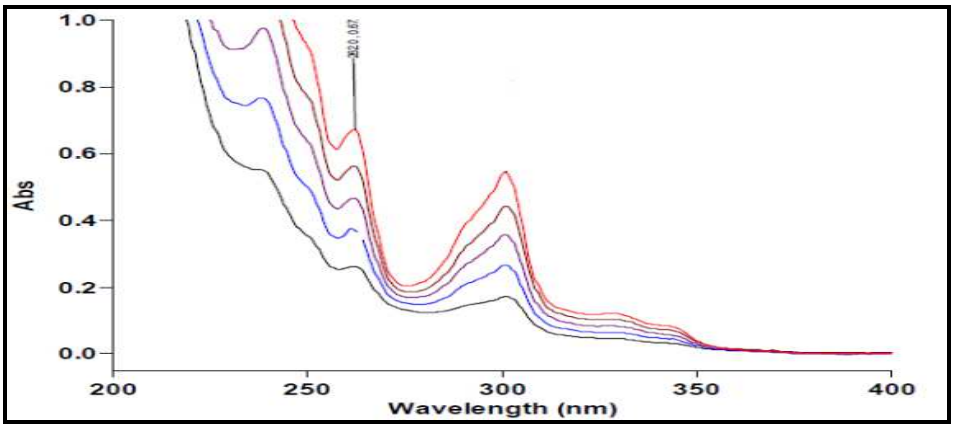

Figure No.3: Overlay Spectra for Carprofen

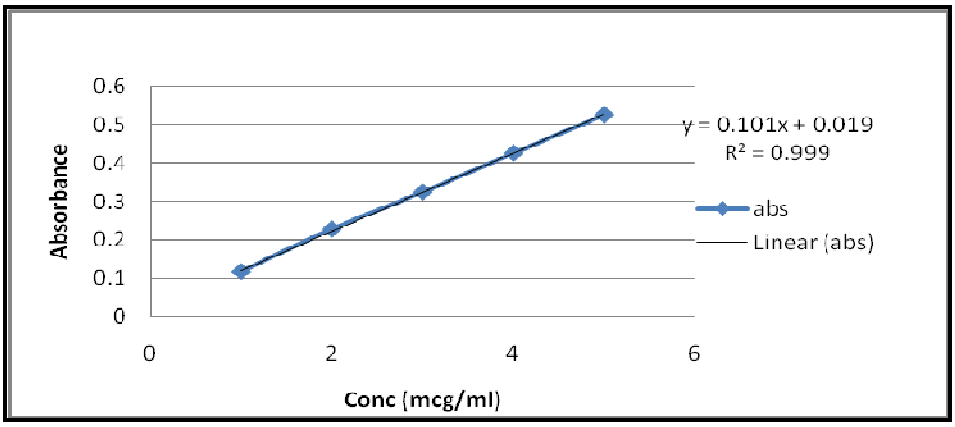

Figure No.4: Calibration curve by method A

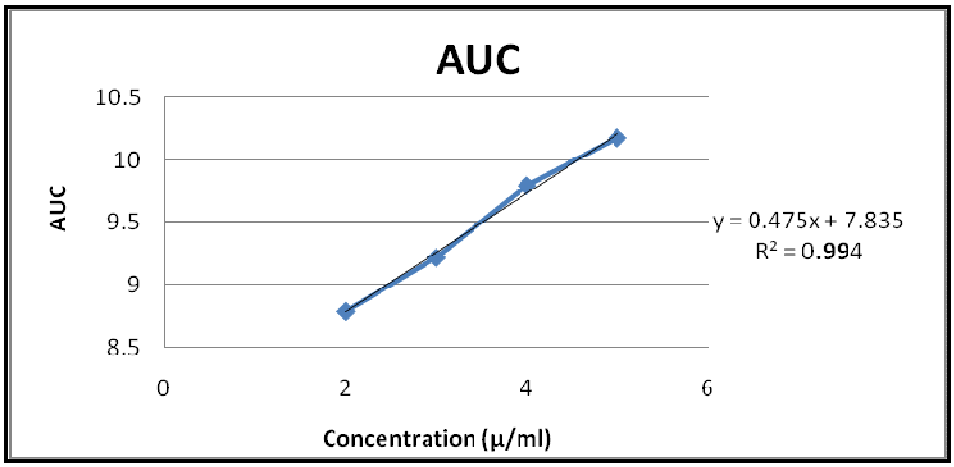

Figure No.5: Area under curve by method B

CONCLUSION

The result of our study indicated that the proposed UV spectroscopic methods are simple, rapid, precise and accurate. Statistical analysis proves that, these methods are repeatable and selective for the analysis of carprofen. It can therefore concluded that these method can save much time with accuracy in an analysis and may be financially as it reduces cost and save money and it can used in laboratories with accuracy.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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