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Development Of New Analytical Method For The Determination Of Bromfenac In Bulk And Marketed Formulation And Its Validation

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Abstract: Method is based on the reaction involving the formation of violet color complex between Bromfenac and 0.015% crystal violet in the presence of 0.5% CAS and 4M H_2SO_4, which obeyed Beer's law in the concentration range of 4-20µg/ml. The method has been validated according to ICH Guidelines for linearity, sensitivity, precision, accuracy, robustness and ruggedness.
Key Words: Bromfenac, Ceric ammonuim sulphate, crystal violet.

Introduction:

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge for chemical substances came from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample[1].

Bromfenac (Figure No. 1) is chemically 2-[2-amino-3-(4- bromobenzoyl)phenyl]acetic acid. It is a Non-steroidal anti-inflammatory drug (NSAID) for ophthalmic use which has the ability to block prostaglandin synthesis by inhibiting cyclooxygenase 1 and 2 (COX-1 and -2)[6a,6b]. It has been evaluated through literature survey that several methods were reported for determination of Bromfenac by HPLC method, RP-HPLC method and UV spectrophotometry[8,9,10,11,12]. In this paper we have reported a new colorimetric method for the assay of Bromfenac. It is simple, accurate and reproducible method.

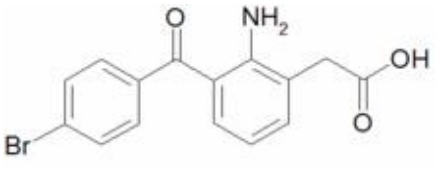

Figure No.1: Bromfenac

Instruments:

(1) The instrument used for the present study was PC based Jasco V-630 UV-Visible double beam Spectrophotometer with 1 cm matched pair quartz cell and spectral bandwidth of 1.5 nm.
(2) SHIMADZU UV-Visible 1700spectro photometer.

Materials:

Pure drug and commercial formulations were procured from Indoco Remedies Pvt. Ltd., Goa. All the chemicals used were of analytical grade.

Reagents:

- Ceric ammonium sulphate (CAS)
- Sulphuric acid
- Crystal violet
- Methanol
- Distilled water

Experimental:

Preparation Of Reagents And Solutions:

- **Preparation of standard stock solution of Bromfenac (1000µg/ml):** Stock solution of Bromfenac was prepared by accurately weighing 100mg of pure drug into a 100 ml volumetric flask and dissolve it in a 25 ml of methanol and the volume was made up to the mark with methanol to get a concentration of 1000 µg/ml (stock solution A).
- **Preparation of working standard solution of Bromfenac (100µg/ml):** The working standard solution of Bromfenac was prepared by pipetting out 10 ml of the standard stock solution into a 100 ml volumetric flask and the volume was made up to the mark with methanol to get a concentration of 100µg/ml (stock solution B). This solution was used for further work.
- **Preparation of 0.5%Ceric ammonium sulphate:** Weigh accurately 0.5mg of Ceric ammonium sulphate (pure) in a 100ml volumetric flask, to this add approximately 1ml of concentrated sulphuric acid drop wise to dissolve the powder and make up the remaining volume up to the mark with distilled water.
- **Preparation of 4M H_2SO_4:** 21.6ml of concentrated H_2SO_4 was taken in a measuring cylinder and was carefully transferred into a 100ml volumetric flask gradually distilled water was added to the flask to make up the volume.
- **Preparation of 0.015% of crystal violet:** 100mg of Crystal violet (pure form) was weighed accurately and transferred to a 100ml volumetric flask and sufficient distilled water was added to dissolve initially. Later the volume was made up to the mark with distilled water and 15ml from the above solution was pipette and transferred to a 100ml volumetric flask and the volume was made up to 100ml with distilled water.

Method:

From the standard working solution ranging from 0.4 to 2 ml were transferred into a series of 10 ml volumetric flasks. To each flask 0.5 ml of 0.5% CAS was added, followed by 1 ml of 4M H_2SO_4 and was kept aside for 20min.Then 1.5ml of crystal violet solution was added and the volume was made up to 10ml with methanol. The absorbance of violet was measured at 582 nm against a reagent blank. The results are given in Table no. 1 and spectra is given in Figure no.2.

Table no. 1: Absorbance of Different concentrations of Bromfenac Obeying beer's law

SL No.	Volume of drug taken (100 µg/ml)	Concentration in µg/ml	Absorbance at 582 nm± S.D
1.	0	0	0
2.	0.4	4	0.135±0.010183
3.	0.8	8	0.275±0.013442
4.	1.2	12	0.385±0.004301
5.	1.6	16	0.531±0.016217
6.	2.0	20	0.641±0.009345

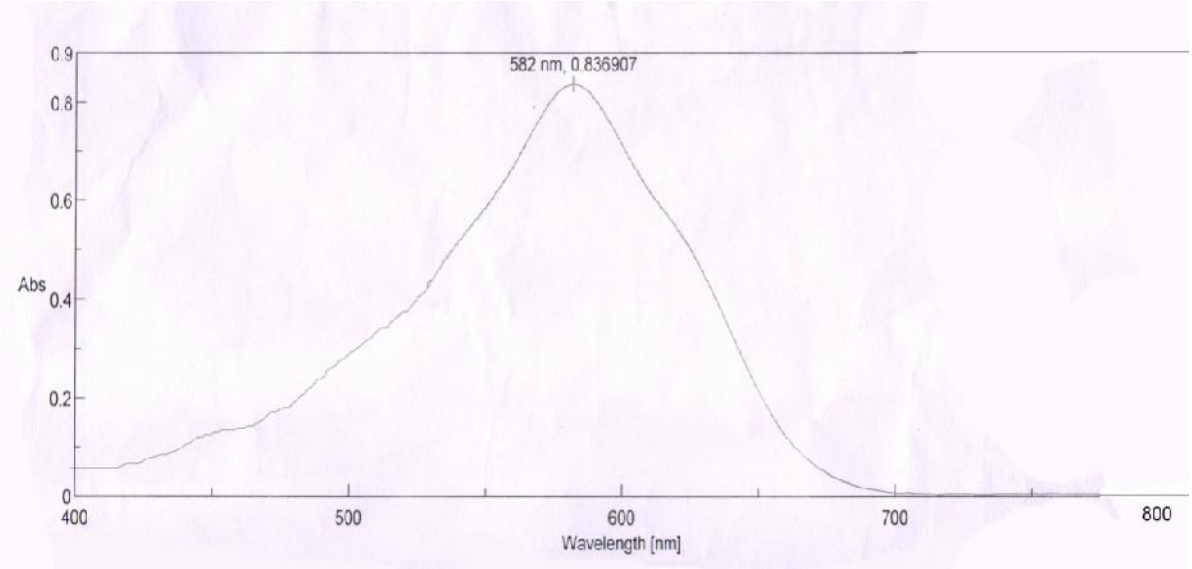

Figure No.2: Absorption spectra of Bromfenac

Validation Of Method:

A. Linearity:

Calibration curve was plotted over a concentration range of 4-20 µg/ml for Bromfenac. Accurately measured standard working solutions of Bromfenac (0.4, 0.8, 1.2, 1.6 and 2.0ml) were transferred to one set of a series of 10 ml volumetric flasks. Then 0.5 ml of 0.5% CAS was added, followed by 1 ml of 4M H_2SO_4 and was kept aside for 20min.Then 1.8ml 0.015% of crystal violet solution was added and the volume was made up to 10m with methanol. The blank was also prepared simultaneously in the same way omitting the drug. The absorbance of the resulting solutions was measured at 582nm against reagent blank.

B. Accuracy(%Recovery):

The accuracy of the methods was determined by calculating % recovery of Bromfenac by standard addition method. Known volumes of standard solutions of Bromfenac were taken for recovery studies in 3 different levels 80, 100, 120% and recovery study was carried out.

C. Method precision (% Repeatability):

The precision of the methods was checked by repeated measurement of the absorbance of standard solutions (n = 6) of 4, 8, 12, 16 and 20µg/ml without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (RSD).

D. Intermediate precision:

The intraday and inter day precision of the proposed methods were performed by analyzing the corresponding responses three times on the same day and on three different days over a period of one week for three different concentrations of standard solutions of Bromfenac (4, 8 and 12 µg/ml).

E. Ruggedness:

To establish ruggedness of the proposed method, assays for two different concentrations of Bromfenac were performed by two different analysts.

F. Reproducibility:

The absorbance readings of 4µg/ ml were measured at different laboratory using different spectrophotometer by another analyst and the %RSD values obtained to verify their reproducibility.

G. LOD and LOQ:

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated by International Conference on Harmonization (ICH) guideline:
LOD = 3.3 X σ/S and LOQ = 10 X σ/S

Where, σ = the standard deviation of the response,

S = slope of the calibration curve.

H. Analysis Of Formulation:

An ophthalmic marketed formulation of Bromfenac ophthalmic solution, 0.09% was procured from Indoco Remedies Pvt. Ltd., Goa. The solution equivalent to 1000µg/ml was taken in 100ml volumetric flask. From that pipette 0.8ml of solution into a 10 ml volumetric flask. Then 0.5 ml of 0.5% CAS was added, followed by 1 ml of 4M H_2SO_4 and was kept aside for 20min.Then 1.8ml 0.015% of crystal violet solution was added and the volume was made upto 10ml. A violet color was obtained. The absorbance was measured against a reagent blank at 582 nm. The result is recorded in Table No. 2.

Table No. 2: Assay Result of Marketed Formulation

Formulation	Actual concentration of Bromfenac (µg/ml)	Amount obtained of Bromfenac (µg/ml)	% Bromfenac
Ophthalmic	8	7.937	99.21%

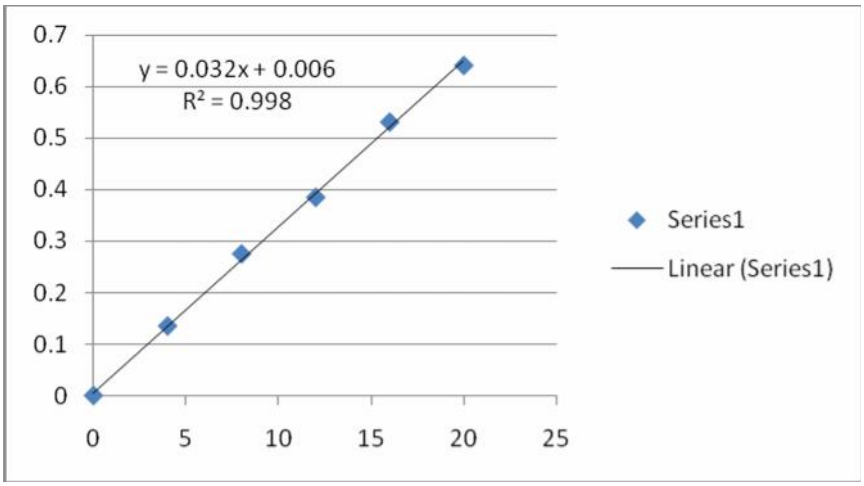

Figure No.3: Standard curve of Bromfenac

Results And Discussion:

Bromfenac was estimated based on the reaction between CAS and crystal violet in the presence of acidic medium. When CAS is added in excess amount, it produces a complex of Bromfenac. Unreacted CAS readily reacts with crystal violet and oxidized crystal violet. Unreacted molecules of crystal violet give color. So, color of the final solution indirectly indicates the amount of drug present. It showed a λmax of 582nm. The color was stable for more than 30 minutes. The method obeyed Beer-Lambert's law in the concentration range of 4-20 µg/ml. The reaction is given in Figure No.4. The method was validated for various parameters like linearity, accuracy, precision and recovery, LOD, LOQ, Ruggedness and Reproducibility.

Table No. 3: Statistical data for Bromfenac at 582nm

Parameter	Result
λmax (nm)	582 nm
Beer's law limits (µg/ml)	4-20 µg/ml
Regression equation (y=a+bc) Slope (b) Intercept (a)	 b=0.032 a=0.001
Correlation coefficient (r^2)	0.998
% recovery	80% = 99.9 100% = 98.71 120% = 99.95
Repeatability (%RSD)	0.30 to0.54
Limit of Detection (µg/ml)	0.0827
Limit of Quantitation (µg/ml)	0.25
Specificity	Specific
Selectivity	Selective
Reproducibility (n=6) Instrument 1 (%RSD) Instrument 2 (%RSD)	 0.14 0.11
Precision (n=3) Intraday precision (%RSD) Interday precision (%RSD)	 0.15-0.43 0.30-0.43

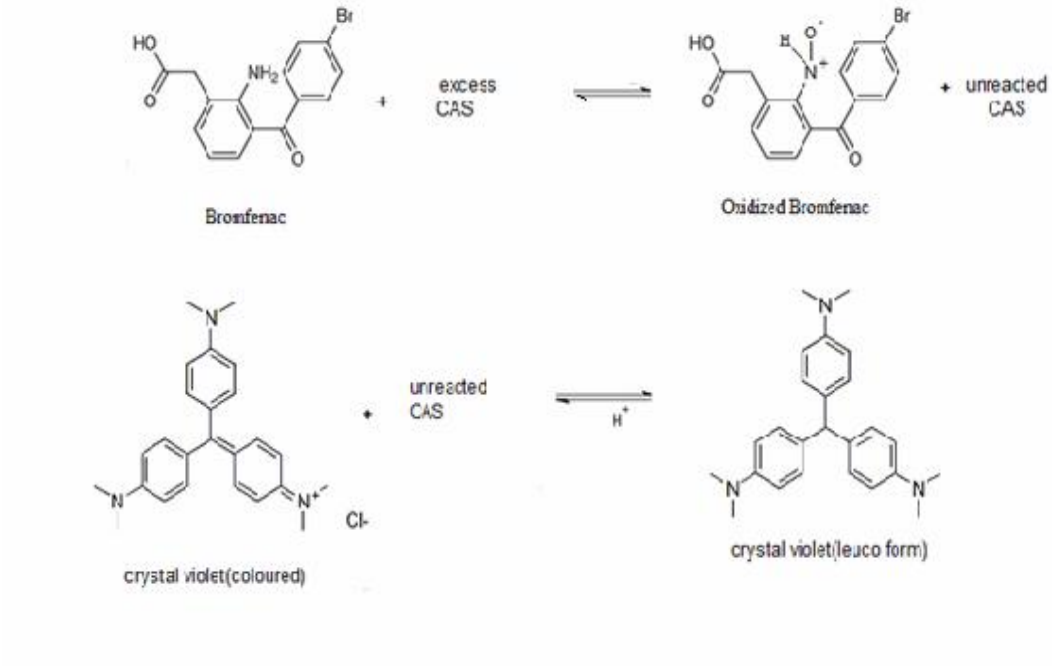

Figure No.4: Oxidation reaction for the development of colour

Conclusion:

The developed visible spectrophotometric method gives sensitive, accurate, precise and economical results for determination of Bromfenac in bulk as well as in pharmaceutical formulation. The most striking feature of these methods is its simplicity and low cost.

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