Research Article

Development of New Method and Validation for Determination of Aceclofenac in Bulk and Marketed Formulation

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ABSTRACT

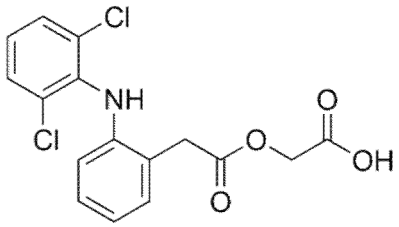
A simple and accurate colorimetric method has been developed for the estimation of Betahistin dihydrochloride in bulk and pharmaceutical dosage forms. Aceclofenac was a colorimetric method based on oxidation-reduction reaction and indirect method. In this method cerric ammonium sulphate has been used for Aceclofenac , in excess amount in presence of sulphuric acid. Remaining amount of oxidizing agents oxidizes standard amount of crystal violet which produces violet color. Absorbance maxima were found to be 585 nm. Linearity range was found 2-18 µg/ml of drug concentration respectively. The method has been validated according to ICH Guidelines.

Keywords: Aceclofenac, Cerric ammonium sulphate, H_2SO_4.

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 of chemical substances comes 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]

Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID). Aceclofenac in combination with paracetamol under the trade name Cincofen. It is used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. The dose is 100 mg twice daily. It should not be given to people with porphyria or breast-feeding mothers, and is not recommended for children.[2] Aceclofenac has higher anti-inflammatory action than conventional NSAIDS. It is a cytokine inhibitor. Aceclofenac works by blocking the action of a substance in the body called cyclo-oxygenase. Cyclo-oxygenase is involved in the production of prostaglandins (chemicals in the body) which cause pain, swelling and inflammation.[3]



Its IUPAC name is 2-[2-[2-[(2, 6-dichlorophenyl) amino] phenyl] acetyl]oxyacetic acid

And formula is $C_{16}H_{13}Cl_2NO_4$. It is soluble in freely soluble in acetone, soluble in alcohol

up to now there is HPLC and spectrophotometric method developed on Aceclofenac.[4-12]

The USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, and Limit of quantitation, Linearity and range, Ruggedness, Robustness [13-14]

EXPERIMENTAL

Aceclofenac was determined spectrophotometrically in bulk and marketed formulation by using crystal violet dye and ceric ammonium sulphate

(CAS) as a strong oxidizing agent in presence of H_2SO_4

1. Preparation of stock solution

Preparation of standard stock solution of Aceclofenac: standard stock solution was prepared by accurately weighing 100 mg of Aceclofenac in 100 ml calibrated volumetric flask and made up the volume with distilled alcohol up to 100 ml.

Preparation of working standard solution of Aceclofenac: working standard was prepared by transferring 10 ml standard stock solution into 100 ml calibrated volumetric flask and made up the volume with distilled alcohol to get Conc. of 100µg/ml.

2. Preparation of reagent

Preparation of 0.5% CAS solution: Weighed accurately 0.5 gm CAS and transferring into 100 ml volumetric flask and made up the volume with distilled water.

Preparation of 4M H_2SO_4: Transferred 216 ml of concentrated H_2SO_4 into 1000 ml volumetric flask and made up the volume with distilled water.

Preparation of crystal violet (0.02%): Weighed accurately 200 mg crystal violet and added in 1000 ml volumetric flask then diluted up to 1000 ml with distilled water.

3. Preliminary investigation

0.5 ml of 0.5% CAS solution, 1 ml of 4M H_2SO_4 and 1 ml of drug solution (100µg/ml) was taken in 10 ml volumetric flask and kept aside for 20 minute for the completion of reaction. 2 ml Crystal violet solution was added in volumetric flask and made up the volume with distilled alcohol. Absorbance against reagent blank was recorded.

3.1 Determination of absorbance maxima

Procedure: 0.5 ml of 0.5% CAS solution, 1 ml of 4M H_2SO_4, 1 ml of the Aceclofenac working standard stock solution was added in 10 ml volumetric flask and kept aside for 20 minutes until the completion of reaction. 2 ml of crystal violet was added and made up the volume with distilled alcohol. Absorbance against reagent blank was recorded. These solutions scanned in UV spectrophotometer range between 400-800 nm. For λmax graph is given in Figure no:1

Model: JASCO V-630
Band width: 1.5 nm
Response: Medium
Measurement: 800-400 nm
λmax: 585 nm
Absorbance: 0.385

4. Investigation

Experiments were carried out to ascertain the optimum concentrations of reagents needed for rapid and quantitative formation of greenish blue coloured species by measuring the absorbance of series of solutions in which one parameter was varied and others fixed.

4.1 Effect of concentration of oxidizing agent (CAS)

0.5 ml different concentrations of CAS solution were taken in 5 volumetric flasks of 10 ml, 1 ml 4M H_2SO_4 and 1 ml of working standard of Aceclofenac were added in each volumetric flask and kept a side for 20 minutes. 2 ml 0.01% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 585 nm and recorded in Table no:1 and Figure no:2

4.2 Effect of volume of oxidizing agent (CAS)

Different volumes of 0.5% CAS solution were added in 5 volumetric flasks of 10 ml. 1 ml 4M H_2SO_4 and 1 ml of working standard of Aceclofenac were added in each volumetric flask and kept aside for 20 minutes. 2 ml 0.01% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 585 nm and recorded in Table no:2 and figures no:3.

4.3. Effect of concentration of H_2SO_4

1 ml different concentrations of H_2SO_4 were taken in 5 volumetric flasks of 10 ml.0.4 ml of 0.5% CAS solution and 1 ml

of working standard of Aceclofenac were added in each volumetric flask and kept aside for 20 minutes. 2 ml 0.01% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 585 nm and recorded in Table no:3 and Figure no:4.

4.4. Effect of volume of 4M H_2SO_4

Different volumes of 4M H_2SO_4 were taken in 5 volumetric flasks of 10 ml. 0.4 ml of 0.5 % CAS solution, 1 ml of working standard of Aceclofenac were added in each volumetric flask and kept a side for 20 minutes. 2 ml 0.01% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 585 nm and recorded in Table No:4 and Figure no:5.

4.5. Effect of concentration of crystal violet

0.4 ml of 0.5% CAS solution, 1 ml of 4M H_2SO_4 and 1 ml of working standard of Aceclofenac were taken in 5 volumetric flasks of 10 ml and kept aside for 20 minutes. 2 ml of different conc. of crystal violet in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 585 nm and recorded in table no:5 and graphs no:6.

4.6. Effect of volume of crystal violet

0.4 ml of 0.5% CAS solution, 1 ml of 4M H2SO4 and 1 ml of working standard of Aceclofenac were taken in 5 volumetric flasks of 10 ml and kept aside for 20 minutes. Different volumes of 0.01% crystal violet was added in each volumetric flask and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 585 nm and recorded in table no:6 and Figure no:7.

4.7 Stability of colour

0.4 ml of 0.5% CAS solution, 1 ml of 4M H2SO4, 1 ml of the Aceclofenac working standard stock solution was added to the 10 ml volumetric flask of and kept aside for 20 minutes. 2.1 ml of crystal violet was added and made up the volume with distilled alcohol. Absorbance against reagent blank was taken at 585 nm for every 10 minutes intervals and recorded in table no:7 and graph is given in figure no:8.

5. Optical characters

5.1. Determination of concentration range

For spectrophotometric analysis determination of the concentration range which obeys the Beer- Lambert's law is necessary for accuracy and reproducibility. It was given from linearity study.

5.2. Preparation of standard curve

Standard curve was prepared by using pure Aceclofenac in the conc. range of 2-18 µg/ml by this method and selecting absorbance maximum at 585 nm.

Reagent and chemicals

1. Working standard stock solution (100µg/ml)
2. 0.5% Cerric ammonium sulphate solution (CAS)
3. 4M H_2SO_4
4. 0.01% Crystal violet
5.

Procedure

0.2, 0.6, 1, 1.4, and 1.8 ml of working standard of Aceclofenac were taken in 6 volumetric flasks of 10 ml. 1 ml of 4M H_2SO_4, 0.4 ml of 0.5% CAS were added in each volumetric flask and kept aside for 20 minutes. 2.1 ml 0.01% of crystal violet solution was added and made up the volume with distilled alcohol. Absorbance was taken against reagent blank at 585 nm and recorded in table no:8 and graph was given in figure no:9

curves for estimation of Aceclofenac

6. Method validation

6.1. Linearity

Linearity was determined over the range of 2-18µg/ml. 0, 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, and 1.8 ml of working standard of Aceclofenac were added in 10 volumetric flasks of 10 ml. 1 ml of 4M H_2SO_4 and 0.4 ml of 0.5% CAS were added in each volumetric flask and kept aside for 20 minutes. 2.1 ml 0.01% of crystal violet solution was added and made up the volume with ethanol. Absorbance was

taken at 585 nm and recorded in table no:8 and graph is given in figure no:9.

6.2. %Recovery (Accuracy)

The accuracy of the methods was determined by calculating % recovery of Aceclofenac by standard addition method. Known volumes of standard solutions of Aceclofenac were taken for recovery studies in 3 different levels 50%, 100%, 150% and recovery study was carried out.

6.3. Method precision (% Repeatability)

The precision of the methods was checked by repeated measurement of the absorbance of standard solutions (n = 6) of 10 µg/ml without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (RSD).

6.4. Intermediate precision (Reproducibility)

The intraday and interday precision of the proposed methods were performed by analysing 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 Aceclofenac (8, 10, 12 µg/ml). The results were reported in terms of relative standard deviation (RSD).

6.5. Limit of detection and Limit of quantification

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 σ/S and LOQ = 10 σ/S

Where, σ = the standard deviation of the response,

S = slope of the calibration curve.

6.6. Analysis of marketed formulation

Aceclofenac is marketed as Aceclof of 100mg tablet manufactured by Cipla were taken for analysis.

Reagent and chemicals

- Working standard stock solution (100µg/ml)
- 0.5% CAS solution
- 4M H_2SO_4
- 0.01% Crystal violet

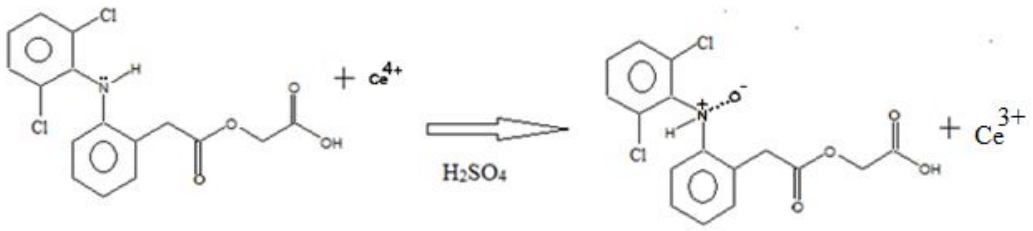
Preparation of sample solution: Aceclofenac tablet powder equivalent to 100 mg was weighed accurately and transferred into 100 ml volumetric flask and made up the volume with distilled alcohol to get 1000 µg/ml Conc. This solution was further diluted to get Conc. of 100µg/ml.

6.7. Recovery experiments

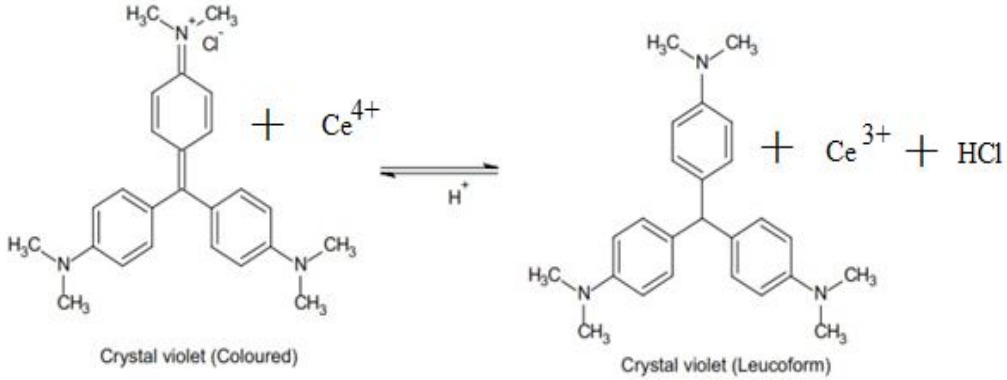
To keep an additional check on accuracy of developed assay method, analytical recovery experiments were performed. The different solutions of different Conc. like 5, 10 and 15 µg/ml were prepared in case of both pure drug solution and formulation extract solution and these solutions were subjected to analysis by the above developed method as mentioned above. The six such samples were prepared and average of that readings taken for calculation of % recovery. This is reported in following table no:9.

RESULT AND DISCUSSION

Carric ammonium sulphate is a strong oxidizing agent. It reacts with Aceclofenac in presence of acidic medium. When CAS was added in excessive amount it oxidises Bronopol and Remaining CAS reacts with crystal violet & crystal violet was oxidised by CAS. Crystal violet which was left after oxidization it produces different colours accordingly which indirectly indicates the amount of drug present.



Aceclofenac reactions with CAS in present of H_2SO_4



Figure

Reaction of crystal Violet with CAS in presence of H_2SO_4

CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analysing huge number of samples in a short time period with due accuracy and precision. A very few analytical method appeared in the literature for the determination of Aceclofenac. In view of the above fact, some simple analytical method was planned to develop with sensitivity, accuracy, precision and economical. In the present investigation, colorimetric method for the quantitative estimation of Aceclofenac in bulk drug and pharmaceutical formulations has been developed.

ACKNOWLEDGEMENT

The author wishes to thanks mates who helped me lot for my work. And how can I forget staff of Srinivas College of pharmacy, Mangalore who suggest me in all way.

Table 1: Effect of Conc. Of CAS for estimation of Aceclofenac

S. No	Conc. CAS in %	Absorbance
1	0.01	0.004
2	0.05	0.02
3	0.5	0.382
4	1	0.106
5	2	0.044

.Table 2: Effect of Volume of 0.5% CAS for estimation of Aceclofenac

S. No	Volume of 0.5% CAS in ml	Absorbance
1	0.2	0.266
2	0.4	0.432
3	0.6	0.225
4	0.8	0.089
5	1	0.005

Table 3: Effect of Conc. Of H_2SO_4 for estimation of Aceclofenac

S.No	Conc. Of H_2SO_4 in molarity(M)	Absorption
1	0.1	0.001
2	1	0.005
3	2	0.084
4	4	0.435
5	6	0.440

Table 4: Effect of volume of 4M H_2SO_4 for estimation of Aceclofenac

S.No	Volume of 4M H_2SO_4 in ml	Absorbance
1	0.5	0.180
2	1	0.436
3	1.5	0.438
4	2	0.440
5	2.5	0.445

Table 5: Effect of Conc. of crystal violet for estimation of Aceclofenac

S. No	Conc. Of crystal violet in %	Absorbance
1	0.0001	0.001
2	0.001	0.012
3	0.01	0.438
4	0.02	0.269
5	0.04	0.152

Table 6: Effect of volume of crystal violet for Aceclofenac

S. No	Volume of crystal violet in ml	Absorbance
1	1.8	0.401
2	1.9	0.443
3	2	0.480
4	2.1	0.521
5	2.2	0.528

Table 7: Stability of colour for Aceclofenac

S. No.	Drug Conc. in µg/ml	Time in minute	Absorbance
1	10	10	0.524
2	10	20	0.524
3	10	30	0.524
4	10	40	0.523
5	10	50	0.523
6	10	60	0.523
7	10	70	0.522
8	10	80	0.522
9	10	90	0.521
10	10	100	0.521
11	10	110	0.520
12	10	120	0.520

Table 8: Standard curves for estimation of Aceclofenac

S. No	Vol. Of working standard drug	conc. of drug (µg/ml)	Absorbance
1	0.2ml	2	0.102
2	0.6 ml	6	0.325
3	1 ml	10	0.522
4	1.4 ml	14	0.729
5	1.8 ml	18	0.912

Table 9: Recovery study for Aceclofenac

Method	Sample	Labelled amount	Amount found	% Recovery
1	Aceclofenac	100 mg	98.79 mg	98.79

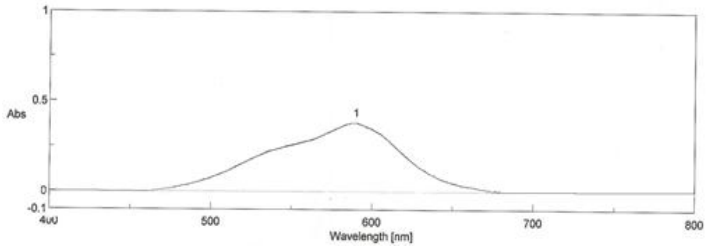
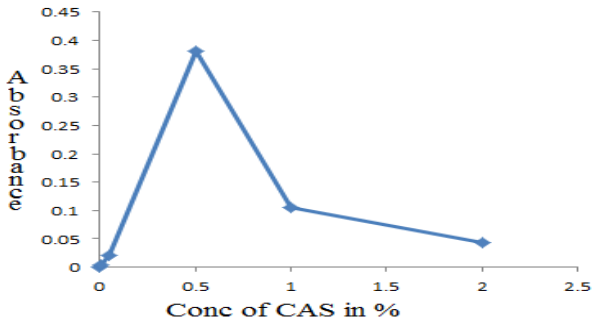
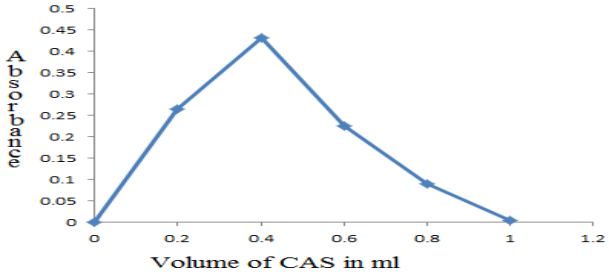


Fig. 1: λmax graph for Aceclofenac



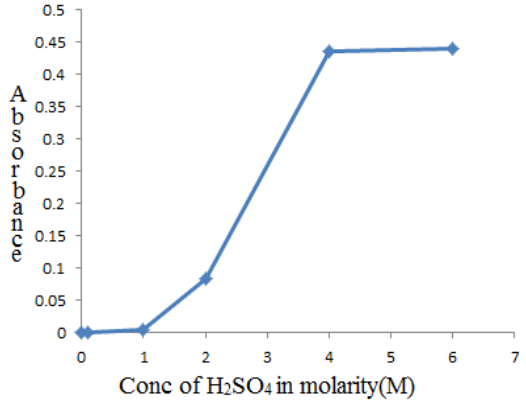
Conclusion: Best absorbance was found in 0.5% CAS solution.

Fig. 2: Absorbance Vs Conc. Of CAS for Aceclofenac



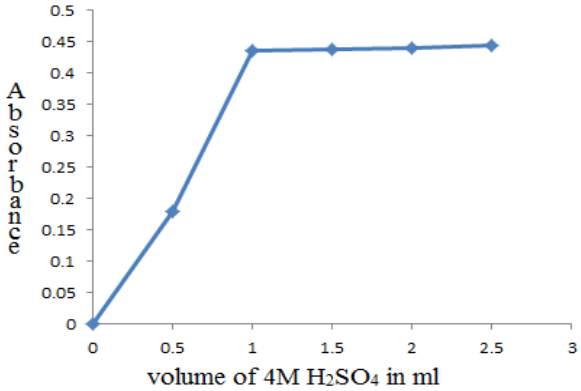
Conclusion: Best absorbance was found in 0.4 mL of 0.5% CAS solution.

Fig. 3: Absorbance Vs Volume of CAS for Aceclofenac



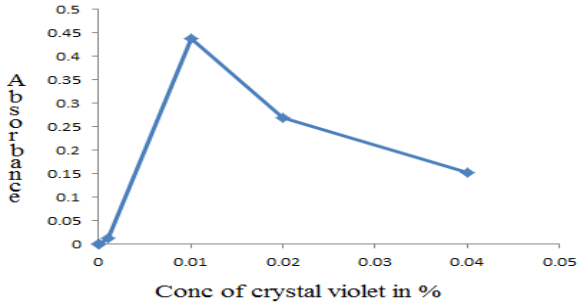
Conclusion: Best absorbance was found in 4M H_2SO_4

Fig. 4: Absorbance Vs concentration of H_2SO_4 for Aceclofenac



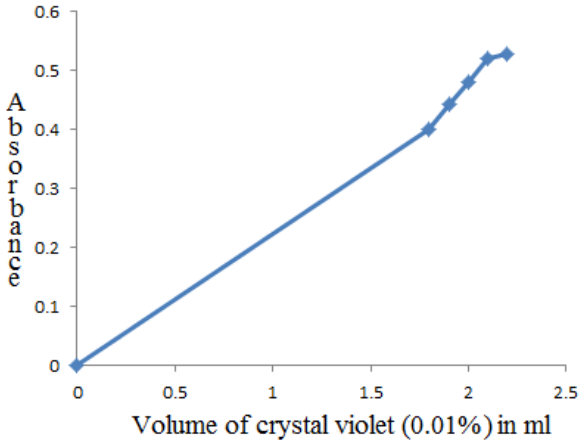
Conclusion: Best absorbance was found in 1 ml of 4M H_2SO_4

Fig. 5: Absorbance Vs volume of 4M H_2SO_4 for Aceclofenac



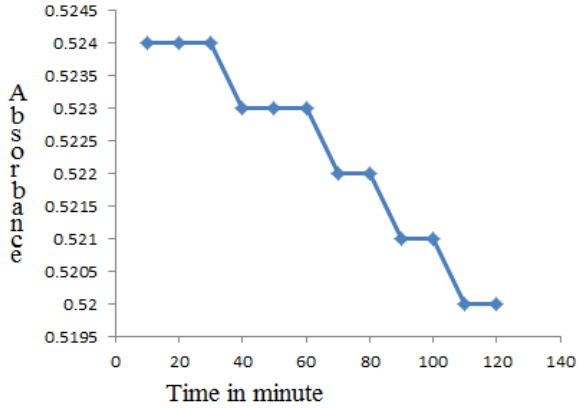
Conclusion: Best absorbance was found in 0.01% conc. of crystal violet solution.

Fig. 6: Absorbance Vs Conc. Of crystal violet for Aceclofenac



Conclusion: Best absorbance was found in 2.1 ml of (0.01%) crystal violet solution.

Fig. 7: Absorbance Vs Volume of crystal violet for Aceclofenac



Conclusion: Stability study of colour was performed and from graph it proved that colour is stable for at least 2 hours.

Fig. 8: Absorbance Vs Time in minute for Aceclofenac

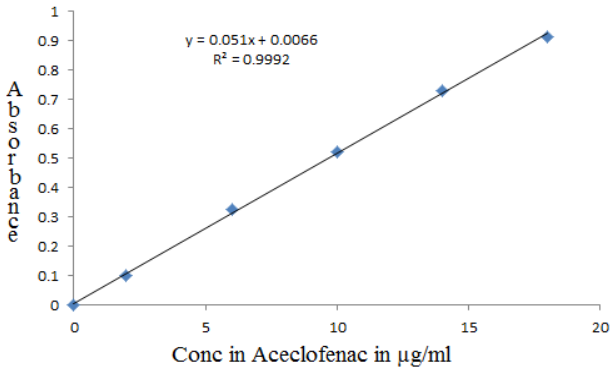


Fig. 9: Standard curves for Aceclofenac

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