MODERNIZED SYNTHESIS OF ACECLOFENAC WITH METHOD ANALYSIS & VALIDATION

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Abstract

Aceclofenac chemically called as 2-[(2,6-dichlorophenyl) amino] phenylacectoxyacetic acid. The aim of the article is to represent the modernized method for the preparation of aceclofenac as the ancient method consume extremely long time to synthesize aceclofenac so modernized method is developed for the synthesis of aceclofenac which is time saving and high yielding. UV spectrophotometric techniques are employed for the method analysis and validation. Titration method, ordinary method, and modified method were all devised for the analysis of Aceclofenac; the modified method for analysis uses method A (zero order), B (first order), and C (Area under curve). The absorption maxima were determined to be 274.65nm for method A (zero order), 259nm for method B (first order derivative), and measured between 269 and 279nm for method C (area under curve).

Keywords: Aceclofenac, COX 2 inhibitor, NSAIDS, modernized.

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INTRODUCTION

2-[(2,6-dichlorophenyl)amino] enylacectoxyacetic acid i.e., aceclofenac is the derivative of diclofenac. The molecular weight of Aceclofenac is 354.19g/mol. Based on the characteristics including permeability and solubility, the Biopharmaceutical Classification System (BCS) categories pharmacological substances into four distinct classes hence aceclofenac comes under Class II in BCS this means poor solubility but have significant permeability. Aceclofenac is used in the pain management. Along with its analgesic and antipyretic actions, aceclofenac also contains anti-inflammatory action. Aceclofenac has good solubility in acetone and ethanol. Aceclofenac in solution state is destroyed when subjected to hydrolytic (acidic, neutral, and alkaline) stresses and light, in contrast to its stability in the solid state against oxidative, photolytic and thermal challenges.Aceclofenac works by blocking the effect of a chemical messenger named as cyclo-oxygenase (COX)enzyme that makes another chemical 'prostaglandins' (PG). For the management of inflammation and pain prostaglandins play vital role. Low number of PGs are produced by blocking the effect of COX enzymes. Due to the lesser number of PGs are produced this helps to reduce inflammation and pain at the damaged area by decreasing the stiffness of joints.AC was developed as an analog of Diclofenac in 1991 through chemical modification; the purpose was to increase the GI, tolerability of the drug.

Acetaminophen's efficacy comes from its ability to inhibit the inflammatory enzymes cyclo oxygenase (COX)-1 and COX-2. Without a steady supply of COX-1 enzyme, the stomach mucosa cannot be protectedand prostacyclin cannot be synthesized. Cyclooxygenase-2 (COX-2) is an enzyme that is essential in the synthesis of inflammatory mediators and may be activated by a variety of triggers. AC is most selectively active towards COX-2 thanCOX-1enzyme.Because of that AC is most GI tolerance than other NSAIDs. The primary metabolite of AC i.e. The COX-2 inhibitory effects of 4'-hydroxyaceclofenac are similar to those of aspirin.

When taken orally, AC is absorbed into the circulation without being altered in any way. The plasma peak conc. after post-ingestion of the drug reaches 1.25 to 3 hrs. The bioavailability of the drug is approx 60% inplasma after penetrating to the synovial fluid. Since maximum concentration (C max) and maximum elimination half-life (T max) are dose-independent, no tolerance or buildup develops.

MATERIALS AND METHODS

The synthesis of Aceclofenac involves two steps. The first step of the process gives the intermediate tert-Butyl- 2-[(2,6-Dichlorophenyl) Amine] phenyl acetoxy acetate which is further utilized to make the final product chemical named as 2-[(2,6-dichlorophenyl) amino] phenyl acectoxyacetic acid. The required amount of 2-[(2,6-Dichlorophenyl) amine] phenylacetic Acid was weighed and mixed with 300ml of tetrahydrofuran (THF). The particles of 2-[(2,6-Dichlorophenyl) amine] phenylacetic Acid were suspended with THF at room temperature. After that 58ml of di-isopropyl ethylamine was added accurately in the conical flask containing the suspending molecules of 2-[(2,6-Dichlorophenyl) amine] phenylacetic Acid with THF. The mixture was stirred continuously until it becomes clear. Further in the process 55ml of tert-Butyl-bromoacetate were added in the mixture when it became clear. The reaction mixed was then heated for 3-4 hours at a room temperature of 40ºC-60ºC. After the competition of reaction, the reaction mixture was basified by adding 30% of NaOH solution dropwise. The basification of the reaction mixture leads to the separation of two layers in the reaction mixture. The organic layer was then patched over a drying agent i.e., Sodium Sulphate. The organic layer was then clarified with the help of petroleum ether. Hence the intermediate tert-Butyl-2-[(2,6-Dichlorophenyl) Amine] Phenyl acetoxy acetate was formed through this process.

In the next step 10g of tert-Butyl-2-[(2,6-Dichlorophenyl) Amine] Phenyl acetoxy acetate was weighed a placed into a conical flask containing 1:1 of trifluoroacetic acid and dichloromethane. The reaction mixture was then stirred well for at least 20-40 min at a room temperature of 15ºC to20ºC. Though the process of stirring the reaction mixture reacts well and after that the reaction mixture was filtered off. To the left-over part in the beaker swater was added to precipitate out the product. The precipitate product obtained was then recrystallized. For recrystallization the solvent ethanol that is used in the process was heated near its boiling point and then added into the beaker having precipitate, further the beaker was place in the ice water for cooling and the pure crystals were formed. Hence the product 2-[(2,6-thedichlorophenyl) amino] phenyl acetoxy acetic acid was filtered and scraped off from the filter paper to the petri dish and dried in the hot air oven.

METHOD ANALYSIS OF ACECLOFENAC

For method Analysis two methods were used i.e Analysis by Titration method and Analysis by UV

Spectroscopic method which further includes ordinary and modified method. For the method analysis of Aceclofenac by titration method 0.3 g of aceclofenac was weighed and dissolved in 40 ml methanol and volume was made 100 ml by distilled water. The volumetric flask labelled as Aceclofenac solution. About 10 ml of Aceclofenac solution was pipette it in a conical flask, add some drops of phenolphthalein indicator and shake and titrate it against 0.1M Sodium hydroxide solution. The end point is the light color of the solution and 3 readings were taken out. 1 ml of 0.1 M sodium hydroxide is equivalent to 0.03542 g of $C_{16}H_{13}Cl_2NO_4$.

For the analysis of Aceclofenac by UV Spectroscopic ordinary method the solutions was diluted to 20 mcg. About 2ml of standard Aceclofenac solution was taken and to this 50ml of methanol and distilled water was added. The dilution was prepared for the UV followed the range of 200-400nm. Six readings were taken with the same dilution. For analysis of Aceclofenac by UV Spectroscopic modified method three methods are used namely Method A (Zero order), Method B (First derivative method) and Method C (AUC method). For method A i.e Zero order spectroscopic method Aceclofenac aliquots from the standard stock solution. 0.5 to 4.0 ml of aliquots with 5 to 40 µg/ml were prepared. 5µg/ml dilution was prepared by pipette 0.5ml of stock solution of Aceclofenac in a 10ml volumetric flask and then volume was made up with the help of distilled water. The same method was repeated till the dilution 4µg/ml Blank reading was taken first with the help of distilled water and then the prepared dilution was scanned at a wavelength of 274.6 nm. For method B i.e First order derivative method the aliquots of 5 to 40 µg/ml was taken same procedure was followed but scanned at the range of 259 nm. For Method C i.e Area under curve method the aliquots of Aceclofenac were taken of the same concentration as above followed by scanning at different wavelength i.e., 269-279nm.

VALIDATION OF METHODS

For validation the methods used are linearity, precision, interlay precision, intraday precision, robustness and ruggedness, accuracy, limit of detection and limit of quantification are used. For linearity drug's reaction was determined to be linear over the studied range, and the linear regression equation and correlation coefficient were established: Method A: y=0.022x+0.005 Correlation Coefficient:0.998 ,Method B: y=0.022x+0.001 Correlation Coefficient:0.999, Method C: y=0.022x+0.001 Correlation Coefficient:0.999The precision study was studied by applying the formula: %RSD=SD/Mean X100.For accuracy reliability of the approach is calculated in three stages using a conventional adding method. The spiked levels are taken as 80,100,120% and added to the sample. The accuracy was indicated by the % recovery studies by applying the formula: % Recovery = conc. (spiked sample)-conc. (µg/ml)/Conc. (added) X100.For validation method limit of quantification (LOQ) and limit of detection (LOD)the formula used was as LOQ=S.D X3.3/Slope and LOD=S.D X10/Slope

RESULTS & DISCUSSION

Aceclofenac chemically named as 2-[(2,6-dichlorophenyl) amino] phenyl acetoxy acetic acid was successfully prepared by the modernized method. The formula for the calculation of the percentage yield is as follows: Actual Yield / Theoretical yield X 100

The percentage yield of 2-[(2,6-dichlorophenyl) amino] phenylacetoxyacetic acid was found to be 88.1%.

- The observations of Aceclofenac by titration method are as follows:

S.No.	Initial reading of burettecontaining titrant (0.1 M NaOH)	"Final reading of burettecontaining titrant (0.1 M NaOH)"	"Volume of 0.1 MNaOH used" (Final reading – Initial reading)
1.	"0 ml"	"10.3 ml"	"10.3 ml"
2.	"0 ml"	"10.1 ml"	"10.1 ml"
3.	"0 ml"	"10.5 ml"	"10.5 ml"

The percentage purity of Aceclofenac was found to be 100.03% by titration method.

- The result of Aceclofenac by UV Spectroscopy method is as follows:

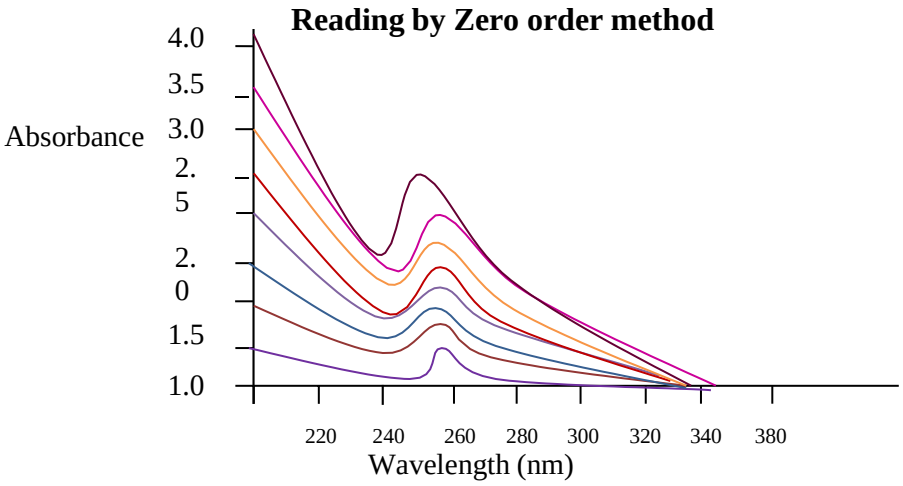
S.No.	Concentration(µg/ml)	Standard absorbance at 275 nm	Test absorbance at 275 nm
1.	2	0.593	0.562
2.	2	0.690	0.663
3.	2	0.642	0.687
4.	2	0.529	0.695
5.	2	0.623	0.598
6.	2	0.583	0.679

The assay of Aceclofenac was found to be 99.72% in λ max 275nm by ordinary method. The result of Assay

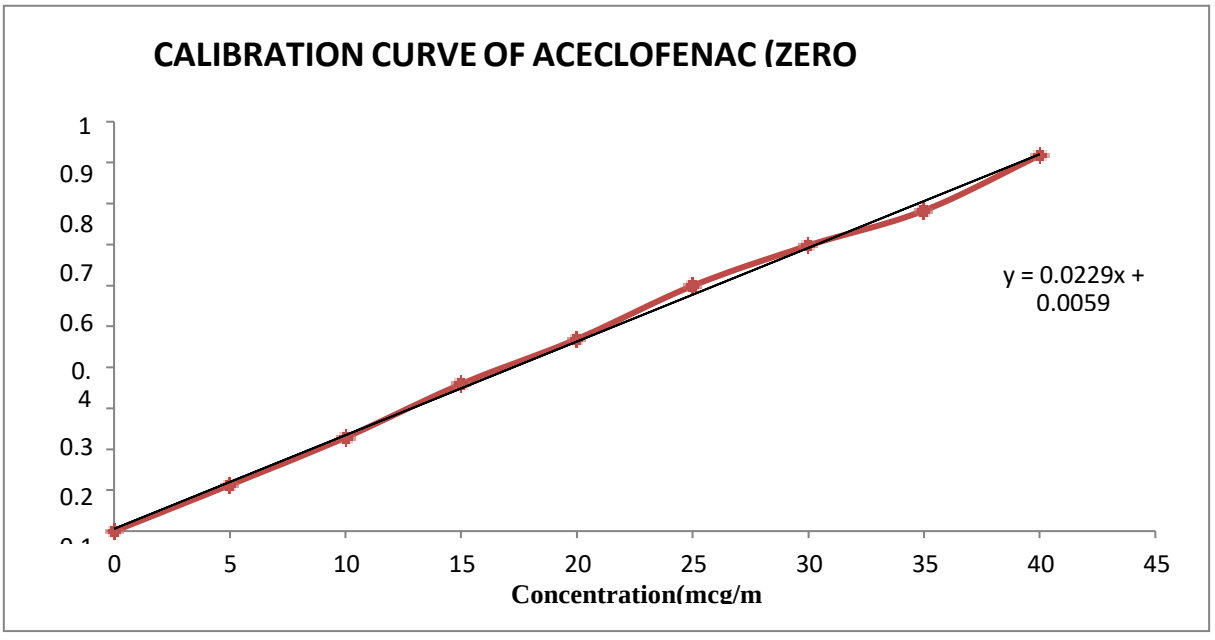
of Aceclofenac by UV Spectroscopy(Modified Method) is following:

Method 1: Overlay Spectrum of Aceclofenac byZero Order Spectrometric Method

S.No.	Conc. (µg/ml)	Absorbance at 274.6 nm
1.	"5"	0.112999
2.	"10"	0.229009
3.	"15"	0.359432
4.	"20"	0.469321
5.	"25"	0.599264
6.	"30"	0.697708
7.	"35"	0.783188
8.	"40"	0.91794



Spectrum by zero order method



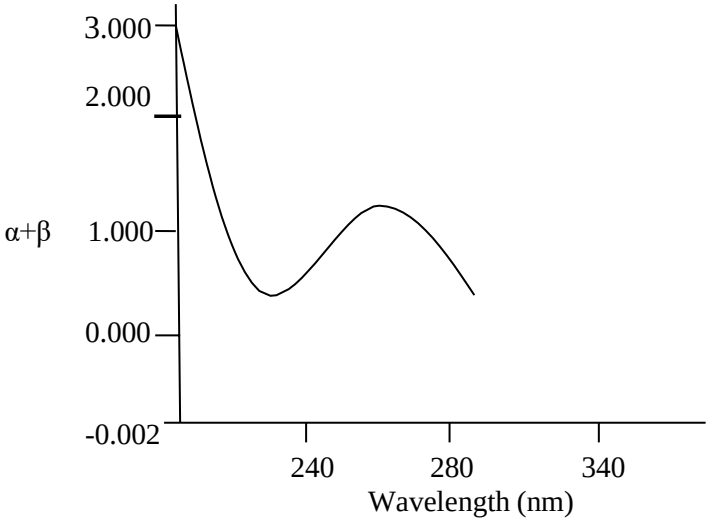
Calibration curve of Aceclofenac Zero-order

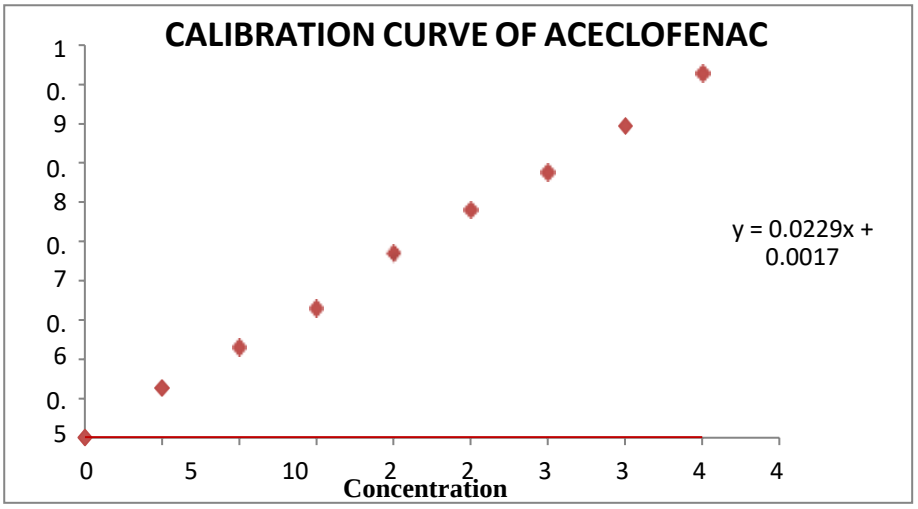
Method 2: Overlay Spectrum of Aceclofenac by First Order Spectrometric Method

S.No.	Conc. (µg/ml)	Absorbance at 259 nm
1.	"5"	0.121989
2.	"10"	0.239007
3.	"15"	0.339432
4.	"20"	0.469217
5.	"25"	0.579264
6.	"30"	0.675708
7.	"35"	0.794188
8.	"40"	0.927946

Method 3: Overlay Spectrum of Aceclofenac byAUC Method

S.No.	Conc. (µg/ml)	Absorbance at 269-279 nm
1.	"5"	0.125989
2.	"10"	0.229007
3.	"15"	0.329432
4.	"20"	0.469217
5.	"25"	0.579264
6.	"30"	0.675708
7.	"35"	0.794188
8.	"40"	0.927946





- The results for the validation methods of Aceclofenac are as follows:

LINEARITY

The proposed UV spectroscopic method's linearity was evaluated by plotting absorbance versus analyte concentration. The Beers law obeyed the method at the conc. range of 5-40µg/ml.

The correlation coefficient values were found to be" 0.9990, 0.9990, and 0.9990 respectively. All the results of linearity given in table below:

S.No.	Parameter	Method A "(Zero order)"	Method B "(First order)"	Method C"(AUC method)"
1.	Linearity(µg/ml	"5-40 µg/ml"	"5-40 µg/ml"	"5-40 µg/ml"
2.	Slope	0.022	0.022	0.022
3.	Intercept	0.005	0.001	0.001
4.	Correlation Coefficient	0.9980	0.9990	0.9990

PRECISION

Analytical precision is the degree to which individual test results are consistent across multiple samples of the same type. The study's results, reported in the form of a coefficient of variation, can be taken as a measure of random

error (CV).
The percent relative standard deviation was used to characterize the accuracy of the methodology (RSD). Findings from studies of weekly and daily variations demonstrated the strategies' validity.

S.No.	Parameters	Method A		Method B		Method C	
		SD	%RSD	SD	%RSD	SD	%RSD
1.	Precision	0.015355	1.48069	0.012237	1.17675	0.015631	1.50490
2.	Intraday precision	0.018660	1.80501	0.017509	1.69297	0.017616	1.70437
3.	Interday precision	0.016006	1.55279	0.016251	1.56538	0.012867	1.24236
4.	Robustness	0.013964	1.36061	0.014414	1.38663	0.015460	1.49118
5.	Ruggedness	0.015137	1.46084	0.014301	1.38207	0.015172	1.46090

ACCURACY

Accuracy is defined as how closely the test results the technique produces resemble the actualvalue. To conduct recovery tests, three different standard drug solution concentrations—80%, 100%, and 120%—were added to the sample solution. Six analyses were performed on each dilution, and the amount of medication recovered was calculated.

S.No.	Conc. (µg/ml)	Spikedlevel (%)	Added amount(mg)	"Amount found (mg)"			"% Recovery"		
				A	B	C	A	B	C
1.	10	80	8	17.97	18.02	17.99	99.62	100.25	99.87
2.	10	100	10	19.93	20.07	20.03	99.30	100.70	100.30
3.	10	120	12	21.99	22.03	21.96	99.91	100.25	99.66

LOD & LOQ VALUES

S.No.	Method	LOD value	LOQ value
1.	A	6.979	2.303
2.	B	5.562	1.835
3.	C	7.105	2.344

OPTICAL CHARACTERISTICS OF ACECLOFENAC

S.No.	Parameters	Method A	Method B	Method C
1.	"Absorption maxima (λmax)"	274.6nm	259nm	269-279nm
2.	"Beers law limit"	"5-40 µg/ml"	"5-40 µg/ml"	"5-40 µg/ml"
3.	"Regression" "equation"	"y=0.022x+0.005"	"y=0.022x+0.001"	"y=0.022x+0.001"
4.	Slope	0.022	0.022	0.022
5.	Intercept	0.005	0.001	0.001
6.	"Linearity indicated bycorrelation coefficient"	0.998	0.999	0.999
7.	Accuracy indicated by % Recovery	99.30-99.91	100.25-100.70	99.87-100.30
8.	Precision(%RSD)	1.48069	1.17675	1.50490
9.	"Intraday precision(%RSD)"	1.80501	1.69297	1.70437
10.	"Interday precision(%RSD)"	1.55279	1.56538	1.24236
11.	Robustness	1.36061	1.38663	1.49118
12.	Ruggedness	1.46084	1.38207	1.46090
13.	LOD	6.979	5.562	7.105
14.	LOQ	2.303	1.835	2.344

CONCLUSION

This paper has presented the process to make the drug Aceclofenac governing the modernized methods, which isless time consuming and having the good yield value. Two steps have been considered in the process in which the first step is to make the intermediate tert-Butyl-2-[(2,6-

Dichlorophenyl) Amine] phenylacetoxyacetate and the intermediate then taken in the second step which gives the final product 2-[(2,6-dichlorophenyl)amino]phenyl acetoxy acetic acid. The paper also governing the percentage yield of the product which was calculated by applying the ap It found that the suggested method was accurate, repeatable, simple to use, effective, and sensitive during this inquiry. When using excipients, the procedure showed no signs of interference. Due to their accessibility and the drug's solubility in each kind of solvent, low- cost

solvents were selected. As a result, we used distilled water, ethanol, and methanol as solvents. The study's methodology satisfied the ICH's requirements for validation (International Council for Harmonization). Linearity, precision followed by intraday and interday, robustness and ruggedness, accuracy after% recovery, LOD, and LOQ are among the strategies that have been proven to be reliable. The % RSD figures for all techniques being less than 2 showed the processes' success. The analysis method can be used for typical laboratory analysis.The methods for the used for the assay are titration method, ordinary method and modified method. The modified methods are further classified in three methods :Method A (Zero order),Method B (First order),Method C (AUC method).The calibration curve was plotted for modified methods and validated according to ICH guidelines. The ordinary method shows its absorbance in 275 nm and the modified method absorbance are for method A 274.6nm, method B 259nm, method C 269-279nm.The regression equation, correlation coefficient, and R^2 was calculated for the modified method.

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