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Development and Validation of UV-Spectrophotometric Method for Simultaneous Estimation of Aceclofenac and Pantoprazole in Bulk and Tablet Dosage Forms Using Hydrotropic Solvent

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ABSTRACT

In present research work a new simple, specific, precise, accurate, robust and economical UV-Spectrophotometric method for simultaneous estimation of Aceclofenac and Pantoprazole in bulk and tablets dosage form using hydrotropic solvent was developed and validated. In the present work mixture of 0.1 M sodium bicarbonate solution and 0.1 M urea solution was used as a hydrotropic solvent to increase the solubility of poorly water soluble Aceclofenac. The analytical wavelengths for Aceclofenac and Pantoprazole are 273nm and 293nm respectively. The developed method was validated as per ICH guidelines in terms of linearity and range, specificity, accuracy, precision, sensitivity and ruggedness. Linearity was obtained in the concentration range of 5-40µg/ml for Aceclofenac and 2-16µg/ml for Pantoprazole with correlation coefficient 0.9998 for both drugs. The % RSD for intraday precision and inter-day precision of Aceclofenac was found to be 0.84 and 1.23 respectively. An intra-day precision and inter-day precision of Pantoprazole was found to be 1.49 and 1.40 respectively. In both cases values were within the acceptance limit of less than 2%. The mean percent recovery for Aceclofenac and Pantoprazole were found to be 98.10% to 99.54 % and 98.66 to 101.33 % respectively. Based on the results obtained the proposed method can be regarded as simple, precise, accurate, reliable, cost-effective and can be used for routine quality control of Aceclofenac and Pantoprazole in bulk and its tablet dosage forms.

INTRODUCTION

Aceclofenac (ACECLO) [1, 2] chemically, a phenylacetic acid derivative, has anti-inflammatory and analgesic properties. It is a Non-steroidal anti-inflammatory drug (NSAIDs) used in various commercial pharmaceutical formulations for the treatment of fever, relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis and reported to have good anti-rheumatic activity. ACECLO is the glycolic ester of Diclofenac. It is inhibitor of cytokine and works by blocking the action of a substance in the body called cyclooxygenase which involved in the production of prostaglandins and responsible for the generation of pain, swelling and their inflammatory conditions. ACECLO is practically insoluble in water and soluble in alcohol & methyl alcohol, freely soluble in acetone & dimethyl formamide. The chemical structure of ACECLO is (Figure 1)

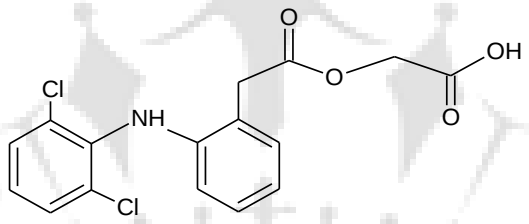

Figure 1: Chemical structure of Aceclofenac

Pantoprazole (PANTO) [1,3] chemically,5-Difluoromethoxyl benzimidazole -2-yl-3,4-dimethoxy-2-pyridyl methyl sulfoxide belongs to the class of substituted benzimidazole and it is a long acting proton pump inhibitor. It acts by suppressing gastric acid secretion by inhibiting H^+ K^+ ATPase at the secretory surface of the parietal cells and blocks the final step of gastric acid secretion. It is more acid stable and has higher bioavailability than omeprazole. It is well absorbed from the Gastrointestinal Tract (GIT). Its bioavailability is 77% and shows dose dependent response with more acid stability and available for intravenous administration, particularly employed in bleeding peptic ulcers. It has lower affinity for cytochrome P450 than Omeprazole or Lansoprazole and has minimal drugs interaction. Pantoprazole is freely soluble in water and very slightly soluble in phosphate buffer pH7.4 and available as white to off-white crystalline powder. The chemical structure of PANTO is (Figure. 2)

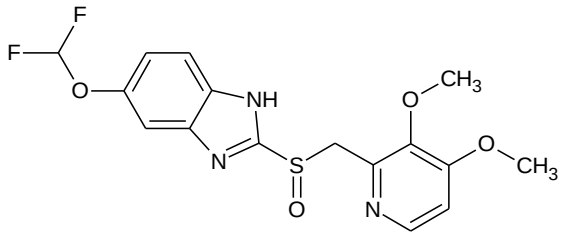

Figure 2: Chemical structure of Pantoprazole

Many pharmaceutical formulations containing ACECLO and PANTO were available in pharmaceutical market in single and combined with other drugs. As the ACECLO is used as NSAID's in treatment of many inflammatory diseases but at same time it causes adverse effect such as gastrointestinal irritation. These common gastric adverse effects can be reduced through suppressing acid production by concomitant use of proton pump inhibitors. Hence to minimize these side effects ACECLO is prescribed along with PANTO as a therapeutic combination. But there was no combined dose formulation of ACECLO and PANTO was available in market. Hence there is lot of gap between formulation development and analytical research. In order to fill this gap an attempt has been made in this research proposal to formulate, evaluate and to estimate the combined dose formulation of ACECLO and PANTO simultaneously using different analytical methods.

Literature survey revealed that various methods such as UV-spectrophotometry [4-6], visible spectrophotometry [7-9], RP-HPLC [10,11], HPTLC [1,12,13] for ACECLO and UV-spectrophotometry [14-16], RP- HPLC [17,18], HPTLC [1] for the estimation of PANTO were reported individually and combination with other drugs. But there was not any UV-Spectrophotometric method developed and validated using hydrotropic solvent for the simultaneous estimation of ACECLO and PANTO in combination. Literature review revealed no method was found for simultaneous estimation of ACECLO and PANTO. Hence there is a need for the development of newer, simpler, rapid, accurate, and reproducible analytical method for simultaneous estimation of ACECLO and PANTO in bulk and pharmaceutical dosage forms. In view of the above facts in the present study, an attempt has been made to estimate the ACECLO and PANTO simultaneously in bulk and pharmaceutical formulations using RP-HPLC.

MATERIALS AND METHODS

Instruments used: Electronic analytical balance; UV-Spectrophotometer, Ultrasonic bath Sonicator were used in the study.

Reagents and chemicals: ACECLO and PANTO standards were obtained as gift sample from Anglo French Drugs and Industries Ltd., Bengaluru. All the chemicals used were of AR grade and are obtained from the stores of Government College of Pharmacy, Bengaluru. ACECLO and PANTO tablet dosage forms were procured from local pharmacy store.

Selection of solvent

By carrying out solubility profile study and literature survey, it was found that ACECLO and PANTO is easily soluble in hydrotropic solvent consisting of 0.1 M sodium bicarbonate and 0.1M urea solution (50:50v/v). Hence hydrotropic solvent was chosen for the UV-Spectrophotometric analysis of ACECLO and PANTO.

Preparation of standard stock solutions:

10 mg of each standard ACECLO and PANTO were weighed separately, into two 10 mL volumetric flasks. Then small amount of hydrotropic solvent was added to dissolve the drugs and then the volume was made up to mark with same to get a concentration of 1 mg/mL of ACECLO and PANTO.

Selection of analytical wavelength

From the above standard stock solutions, 0.1 mL aliquots was taken separately into two 10 mL volumetric flask and diluted up to the mark with hydrotropic solvent and these solutions were scanned in the UV region of 200-400nm. Maximum absorbance was seen at the wavelength of 273 nm for ACECLO and 293nm for PANTO. Hence all absorbance measurements were made at 273nm for ACECLO and 293nm for PANTO.

Calibration Curve:

A series of dilutions were prepared from the standard stock solutions of ACECLO and PANTO to obtain the concentration of 5-40µg/ml of ACECLO and 2-16µg/ml of PANTO. Absorbance of

the above solutions was measured at 273nm and 293nm for ACECLO and PANTO respectively and a calibration curve of absorbance against concentration was plotted and the regression coefficient (R^2) was also determined.

Determination of absorptive coefficients:

The absorptive coefficient of both drugs (ACECLO and PANTO) was determined at selected wavelengths by using the formula: **$A=A\ (^{1\%}_{1}\ cm)\ b\ x\ c.$** Where, c = concentration of the absorbing species, in g/100 mL and b = path length in cm. The absorptivity values are then substituted in the following equations (1) and (2):

$$A_1 = ax1\ Cx + ay1\ Cy \text{…….(1)}$$

$$A_2 = ax2\ Cx + ay2\ Cy \text{…….(2)}$$

Where,

A_1 and A_2 are absorbance of sample at 273nm and 293nm respectively. ax1and ax2 are absorptivities of ACECLO at 273nm and 293nm respectively. ay1 and ay2 are absorptivities of ACECLO and PANTO at 273nm and 293nm respectively. Cx and Cy are concentrations of ACECLO and PANTO respectively.

Preparation of sample solutions

Average weight of twenty tablets containing 100 mg of ACECLO and 40 mg of PANTO (labeled claim) was calculated separately. The tablets were powdered well in glass mortar with pestle. Quantity of powder equivalent to 100 mg of ACECLO and 40 mg of PANTO was weighed accurately and transferred separately into 25 mL volumetric flasks. Then a small quantity of hydrotropic solvent was added and sonicated for 30 minutes to dissolve the drugs completely and then the volume was made up to the mark with hydrotropic solvent and filtered through 0.45 µm membrane filter. From this, 0.1 mL was taken and diluted with hydrotropic solvent. The absorbance of this solution was measured at 273nm and 293nm against hydrotropic solvent as a blank. The assay was performed in triplicate.

Analysis of tablet dosage form

Aliquots portion of the above sample stock solution was diluted with phosphate buffer and the absorbance was measured at appropriate wavelength and the concentration of the two drugs were determined using equations (3) and (4). Analysis was done in triplicate.

$$Cx = (A_2\, ay1 - A_1\, ay2) / (ax2\, ay1 - ax1\, ay2) \ldots\ldots\ldots (3)$$

$$Cy = (A_1\, ax2 - A_2\, ax1) / (ax2\, ay1 - ax1\, ay2) \ldots\ldots\ldots (4)$$

METHOD VALIDATION

The developed UV-Spectrophotometric method was validated as per ICH guidelines [19, 20] in terms of linearity and range, specificity, precision, sensitivity, ruggedness and accuracy.

ln order to determine **Linearity range** of developed method a series of solutions were prepared using ACECLO and PANTO standard stock solutions at concentration range of 5-40µg/ml and 2-16µg/ml respectively. The absorbencies of the resultant solutions were measured at 273nm and 293nm against phosphate buffer as blank. The calibration curves were constructed by plotting concentrations on x-axis and absorbance on y-axis. R^2 value not less than 0.999 was regarded as acceptance criteria.

Specificity was performed to exclude the possibilities of interference of solvent in the region of maximum absorbance peaks of ACECLO and PANTO. The specificity of the method was tested under the normal conditions and results of the tests proved that the components other than ACECLO and PANTO did not produce the detectable peaks at the maximum absorbance peaks of both the drugs.

Accuracy of the developed method was determined by recovery studies at three different levels. The pre-analyzed samples were spiked with 80, 100 and 120 % of mixed standard solution. The mixtures were analyzed and the recoveries were determined. The recovery study was carried out in triplicate. The mean % recovery of the ACECLO and PANTO at each level should not be less than 98.0 % and not more than 102.0% was considered as the acceptance criteria.

Precision was studied to find out intra-day and inter-day variations in the test method of ACECLO and PANTO. Intra-day assay precision was found by analysis of standard drug thrice

on the same day in different intervals of time. Inter-day assay precision was carried out on three different days and percentage relative standard deviation (% RSD) was calculated. The % RSD should not be more than 2.0%.

Sensitivity of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ). The LOD and LOQ of ACECLO and PANTO by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3s/S and 10s/S respectively, where S is the slope of the calibration curve and s is the standard deviation of response.

Ruggedness expresses the variations within the laboratory conditions (different day, different analyst and different instrument. The ruggedness was performed by analyst 1 and analyst 2 on different instruments on different days.

RESULTS

A UV-Spectrophotometric method has been developed for simultaneous estimation of ACECLO and PANTO using hydrotropic solvent. The developed method specifications were presented in **Table 1**. The developed UV-Spectrophotometric method was validated as per ICH guidelines in terms of linearity, specificity, precision, sensitivity, ruggedness and accuracy. The results of validation parameter found to be well within the acceptance limit.

The linearity response of ACECLO and PANTO was observed in the concentration range of 5-40µg/ml and 2-16µg/ml respectively for both the drugs respectively and statistical data such as regression equation and correlation coefficient was found well within the acceptance criteria limit. The results were presented in **Table 2,3 and** The UV spectrum was presented in **Figure 3-6** and standard calibration curve was presented in **Figure 7,8.**The developed method was found to be specific as the solvents used and excipients of tablet formulation showing absorbance at maximum absorbance wavelength of ACECLO and PANTO and not interfere in the analysis and also method was found to be accurate as the accuracy study data of ACECLO and PANTO showed excellent % recovery values at three different levels. The results of accuracy study were presented in **Table 4, 5, 6.**The % RSD value of concentration obtained for six replicates of injections of ACECLO and PANTO was found to be less than 2%. Hence developed method was found to be precise. The developed method was found to be sensitive and rugged and results

were presented in **Table 7.** The results of % assay shows that there is no interference of excipients and no impurities were observed in sample for the developed HPTLC method. The results of assay were presented in **Table 8.**

Table (1): Developed UV method specification

Instrument and Specification	UV-Spectrophotometer Shimadzu 1800
Scanning Range	200 nm to 400 nm
Solvent Used	Hydrotropic Solvent
Strength of Solvent	0.1 M
Composition of Solvent	Sodium bicarbonate solution and urea (50:50 v/v)
Wavelength Maxima of ACECLO	273 nm
Wavelength Maxima of PANTO	293nm

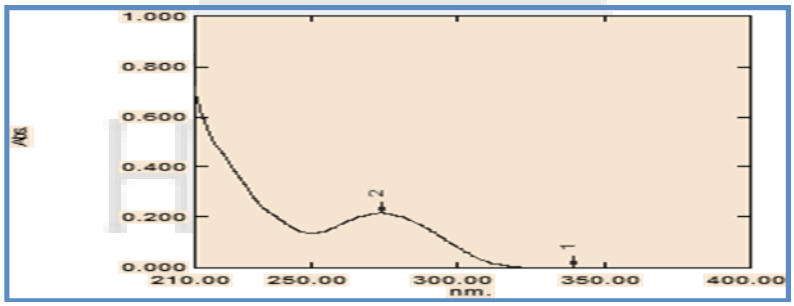

Fig.(3): UV-Spectrums of Aceclofenac

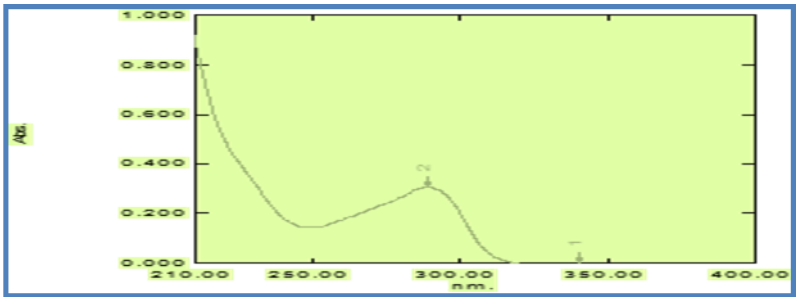

Fig.(4): UV-Spectrums of Pantoprazole

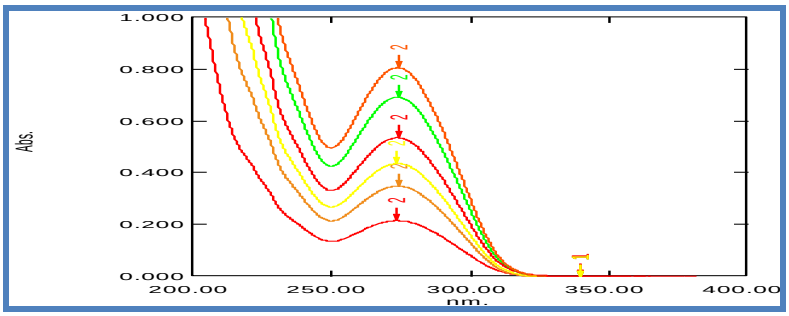

Fig.(5): Overlay Spectrum of Aceclofenac(5-40 µg/ml)

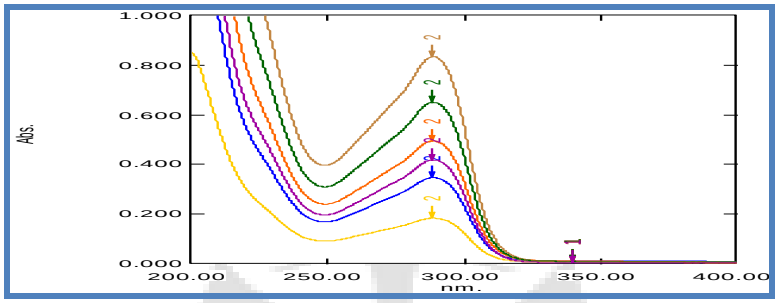

Fig. (6): Overlay Spectrum of Pantoprazole (2-16 µg/ml)

Table (2): Linearity range data of Aceclofenac and Pantoprazole

Drugs	**ACECLO**		**PANTO**	
Sr. No.	**Conc. µg/ml**	**Abs at 273 nm**	**Conc. µg/ml**	**Abs at 293 nm**
1	5 µg/ml	0.110	2 µg/ml	0.112
2	10 µg/ml	0.221	4 µg/ml	0.224
3	15 µg/ml	0.332	6 µg/ml	0.338
4	20 µg/ml	0.445	8 µg/ml	0.452
5	25 µg/ml	0.558	10 µg/ml	0.563
6	30 µg/ml	0.672	12 µg/ml	0.674
7	35 µg/ml	0.783	14 µg/ml	0.791
8	40 µg/ml	0.897	16 µg/ml	0.896

Table (3): Linearity and range report of Aceclofenac and Pantoprazole

Parameters	ACECLO	PANTO
Linearity Range	5-40 µg/ml	2-16 µg/ml
Regression equation	Y=0.0225x-0.0014	Y=0.06x+0.0004
Correlation Coefficient	0.9998	0.9998
Intercept	44612	44612
Slope	0.022	0.056

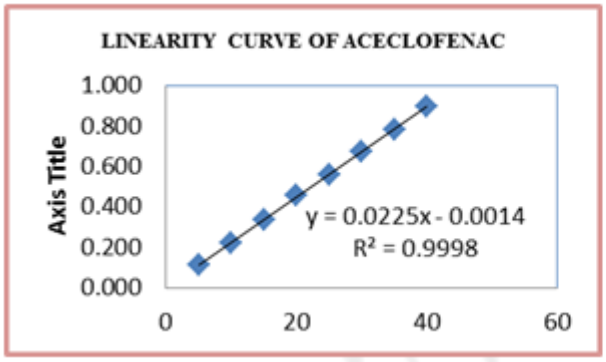

Fig.(7): Standard calibration curve of Aceclofenac

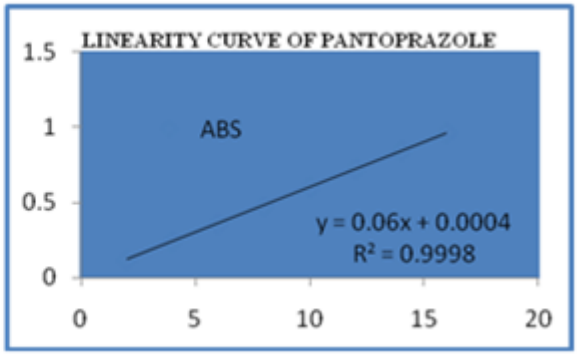

Fig.(8): Standard calibration curve of Pantoprazole

Table (4): Accuracy study data of Aceclofenac

Level	Replicate	Std Conc. (µg/ml)	Sample Conc. (µg/ml)	Conc. found (µg/ml)	std. recovered (µg/ml)	% Recovery
80%	**I**	**3**	10	13.03	3.03	101.05
	II	3	10	13.07	3.07	102.56
	III	3	10	12.98	2.98	99.33
100%	I	5	10	14.84	4.84	96.83
	II	5	10	15.11	5.11	102.2
	III	5	10	14.93	4.93	98.64
120%	I	7	10	17.01	7.01	100.19
	II	7	10	17.10	7.10	101.48
	III	7	10	16.78	6.78	96.96

Table (5): Accuracy study data of Pantoprazole

Level	Replicate	Std Conc. (µg/ml)	Sample Conc. (µg/ml)	Conc. found (µg/ml)	std. recovered (µg/ml)	% Recovery
80%	I	3	4	7.00	3.00	100.00
	II	3	4	6.92	2.92	97.33
	III	3	4	6.91	2.91	97.02
100%	I	5	4	9.00	5.00	100.00
	II	5	4	9.07	5.07	101.42
	III	5	4	9.10	5.10	102.14
120%	I	7	4	11.00	7.00	100.00
	II	7	4	10.92	6.92	98.97
	III	7	4	10.96	6.96	99.48

Table (6): Recovery Studies Report of Aceclofenac and Pantoprazole

Levels	Mean % Recovery of ACECLO	Mean % Recovery of PANTO
80 %	100.98 %	98.11 %
100 %	99.22 %	101.18 %
120 %	99.54 %	99.48 %

Table (7): Summary of the validation parameters of the proposed method

Parameters	**ACECLO**	**PANTO**
Maximum absorbance	273nm	293nm
Linearity	5-30µg/ml	2-16µg/ml
Correlation Coefficient	0.9998	0.9998
Absorptivity at 273nm	222.37	230.06
Absorptivity at 293nm	133.27	561.14
Precision (% RSD) (i) Intra day (ii) Inter day	 0.84 1.23	 1.49 1.40
% Recovery	100.08%	100.51%
LOD	0.47µg/ml	0.05µg/ml
LOQ	1.43µg/ml	0.16µg/ml
Ruggedness	99.0-100.9% **(Analyst-1)** 98.1-101.3% **(Analyst-2)**	99.50-101.75% **(Analyst-1)** 99.0-100.9% **(Analyst-2)**
Tablet Assay	99.54%	101.33%

Table (8): Assay Results of Aceclofenac and Pantoprazole

Drugs name	**Brand name**	**Labeled amount**	**Amount found**	**% assay**
ACECLO	HIFENAC	100 mg	99.54 mg	99.54%
PANTO	PAN 40	40 mg	40.05 mg	101.33%

DISCUSSION

In the present research work a new UV Spectrophotometric method for the simultaneous analysis of ACECLO and PANTO in bulk and commercially available pharmaceutical dosage forms was developed and validated. The spectrum of ACECLO and PANTO in hydrotropic solvent showed

the absorption maximum at 273nm and 293nm respectively. The statistical analysis of data obtained from the calibration curve of ACECLO and PANTO in pure solution indicated a high level of precision for the proposed method, as evidenced by low value of coefficient of variation. The coefficient of correlation was highly significant. The linearity range was observed between 5-40µg/ml for ACECLO and 2-16µg/ml for PANTO. The plot clearly showed a straight line passing through origin. The assay method was validated by low values of % RSD and standard error, indicating accuracy and precision of the methods. Excellent recovery studies further prove the accuracy of the method. The ruggedness of method was studied by using different instrument and different analyst. From the high values of recovery study, it can be inferred that the method is free from excipients used in the formulation. Based on the results obtained the developed method can be regarded as simple, accurate, precise and reliable which can be employed for routine quality control of ACECLO and PANTO in bulk and pharmaceutical dosage forms.

CONCLUSION

The proposed UV-Spectrophotometric method was found to be simple, precise, sensitive, specific and economic for simultaneous estimation of ACECLO and PANTO in bulk and pharmaceutical dosage form with good accuracy and precision. The proposed method utilizes inexpensive solvents and the % recovery data shows that the method is free from interference of the excipients used in formulation and hence can be used for routine analysis in quality control laboratories.

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