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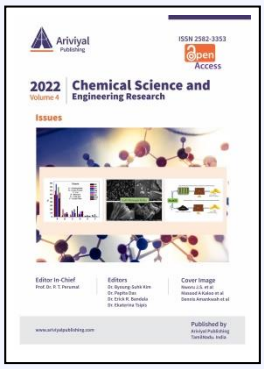

Development and Validation of UV Spectroscopic First Derivative Method for Simultaneous Estimation of Aceclofenac and Serratiopeptidase in Synthetic Mixture

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Abstract: A UV- visible Spectrophotometer method has been developed and validation for Simultaneous estimation of Aceclofenac and Serratiopeptidase in tablet dosage form using double form using double beam UV Spectrophotometer of thermo Electron Corporation with (Methanol + Water) as a solvent. Absorption maxima of Aceclofenac in Methanol and Serratiopeptidase in Methanol diluted with water was found to be 274 nm and 279 nm, respectively. Beer's law was obeyed in the concentration range 10-50 µg/ml for Aceclofenac and 1.5-7.5 µg/ml Serratiopeptidase. The methods allow rapid analysis of binary pharmaceutical formulation with accuracy, precision. The method was found to be simple, accurate, and precise.

Keywords: Aceclofenac; Simultaneous Estimation; Q-analysis; Serratiopeptidase; Isoabsorptive Point

1. Introduction

Aceclofenac (ACE) and Serratiopeptidase (SER) are available in tablet dosage form in the ratio of 100:15 mg. Aceclofenac[1,2,3] is cox-2 inhibitor, chemically (2,6-dicholorophenyl) amino phenyl acetoxy acetic acid used as analgesic & anti-inflammatory agent (Fig. 1). It is used in the treatment of rheumatic disorders and soft tissue injuries. Aceclofenac inhibits the cyclooxygenase enzyme and thus exerts its anti-inflammatory activity by inhibition of prostaglandin synthesis. The European Pharmacopoeia supplement 2000 and the British Pharmacopoeia reported HPLC methods for the determination of Aceclofenac presence of Diclofenac.[4-5] Others methods include titrimetric,[4] electrochemical,[5-7] spectrometric,[6-8-11-12-13] spectroflurometric[6] and chromatographic method.[7-9-10-14]

Serratiopeptidase is an enzyme derived from bacteria belonging to genus *Serratia* sp. Serratiopeptidase is a 'photolytic' or protein digesting enzyme. Serratiopeptidase can help in the removal of dead tissue (such as blood clots arterial plaque), increase circulation and help in the relief of inflammation and strengthening of joints. Serratiopeptidase can help speed up post-operative healing times. Serratiopeptidase can also provide relief from allergy symptoms such as swelling of the nasal passage.

Aceclofenac is Official in BP 2009 while Serratiopeptidase is non-official. Literature survey reveals that no reported methods available for simultaneous analysis of both drugs in combination. Hence an attempt has been made to estimate them simultaneously by Q-analysis method by UV- visible Spectrophotometric analysis. The aim of the present work was to develop a simple, sensitive, accurate, and precise Q-analysis method for routine analysis.

2. Materials and Methods

2.1. Location

This study was conducted at the Department of Chemistry, R.K University, Rajkot, Gujarat state, India.

2.2. Chemicals and Reagents

Standard gift sample of ACE and SER were obtained from Gujarat Terce Lab. Ltd., Gandhinagar. Methanol was used as a solvent in study. Distilled water was used for this study.

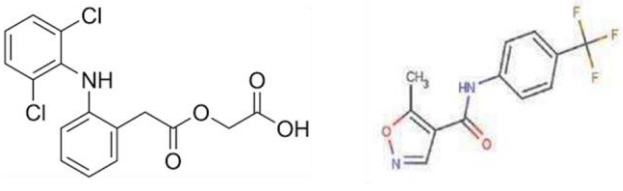

Fig. 1. Structure of Aceclofenac and Serratiopeptidase

Table 1. Data for Standard Calibration Curve of Aceclofenac at 274 nm

Reno	Concentration (µg/ml)	First derivative at 279 nm
1	1.5	0.61
2	3	0.78
3	4.5	0.86
4	6	1.08
5	7.5	1.23

Table 2. Data for Standard Calibration Curve of Serratiopeptidase at 279 nm

Reno	Concentration (µg/ml)	First derivative at 274 nm
1	10	0.075
2	20	0.077
3	30	0.080
4	40	0.083
5	50	0.087

2.3. Instrumentation

UV Spectroscopy was performed with UV software by using UV Visible Spectrophotometer (SHIMADZU UV-1900). An electronic analytical weighing balance used in this study.

2.4. Preparation of Stock Solution

Standard stock solution containing Aceclofenac (ACE) and Serratiopeptidase (SER) was prepared by dissolving 10 mg of Aceclofenac separately in 10 ml methanol and 15 mg of Serratiopeptidase in 10 ml distilled water for dissolution of SER, then add methanol for dilute up to 10 ml with double distilled water to get the stock solution containing 100 µg/ml of Aceclofenac and 150 µg/ml of Serratiopeptidase in two different 10 ml volumetric flask.

2.5. Selection of Analytical Wavelength

1 ml of stock solution of ACE and 1.5 ml of SER from stock solution ware pipette out in 10 ml volumetric flask, diluted up to mark with solvents. Solution containing 10 µg/ml of Aceclofenac and 1.5 µg/ml of Serratiopeptidase were scanned separately in the range of 200-800 nm to determine the wavelength of maximum absorbance for both the drugs. Aceclofenac showed absorbance maxima at 274 nm (Table 1) and Serratiopeptidase showed absorbance maxima 279 nm (Table 2).

2.6. Selection of Analytical Concentration Range

For each drug appropriate aliquots were pipette out from the standard stock solution of ACE into series of 10 ml volumetric flasks. The volume was made up to the mark with methanol to gee a set of solutions having the concentration 10, 20, 30, 40, 50 µg/ml for Aceclofenac (Fig. 2) and 1.5, 3, 4.5, 6, 7.5 µg/ml for Serratiopeptidase (Fig. 3). The absorbance of each of these solutions were measured at the selected wavelengths (for ACE at 274 nm and for SER at 279 nm) and plotted against concentration. The concentration range over which the drugs obeyed Beer's law was chosen (Fig. 4, 5). The range was found to be 10 to 50 µg/ml for Aceclofenac and 1.5 to 7 µg/ml for Serratiopeptidase. The working curve equation was found to be y = 0.003x + 0.071 with a correlation coefficient (r^2) value of 0.986 for ACE and y = 0.235x - 0.097 with correlation coefficient (r^2) value of 0.989 for SERA.

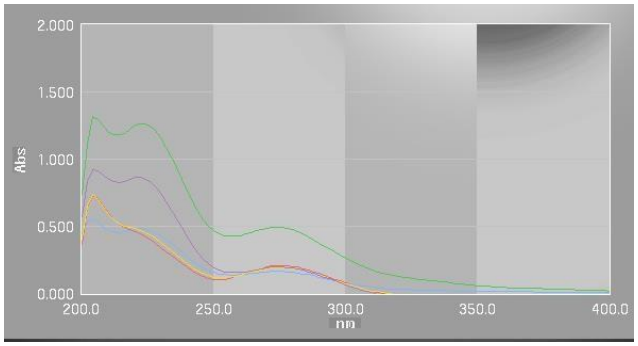

Fig. 2. Overlay spectra of Aceclofenac (10-50 µg/ml)

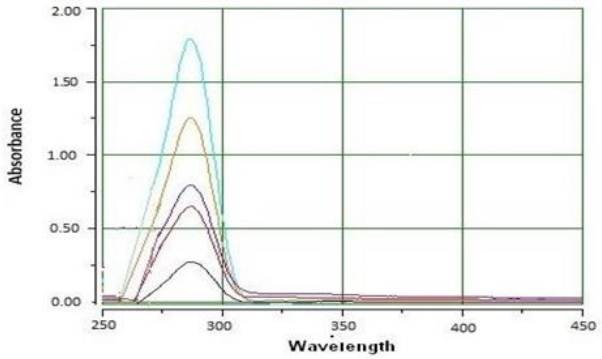

Fig. 3. Overlay spectra of Serratiopeptidase (0.15 - 0.75 µg/ml)

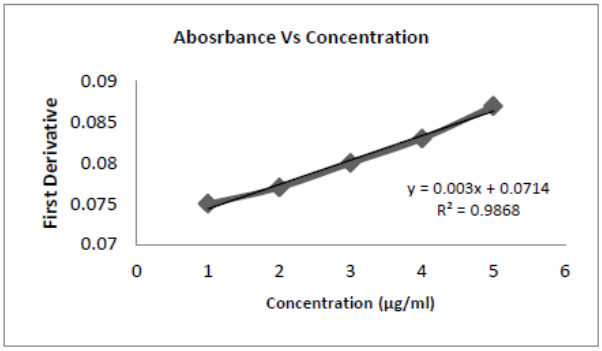

Fig. 4. Calibration curve of Aceclofenac at 274 nm in methanol within the range of 10 to 50 µg/ml the drug obeyed beer's law.

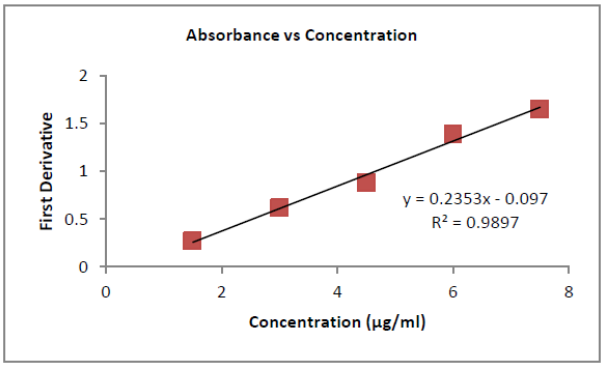

Fig. 5. Calibration curve of Serratiopeptidase at 279 nm in methanol + d.w. within the range of 1.5 to 7.5 µg/ml the drug obeyed beer's law

2.7. Development of First Derivative Method for Aceclofenac and Serratiopeptidase

Concentration of C_{ACE} and C_{SERA} can be obtained from calibration curve (Figs. 4, 5).

Table 3. Statical Validation for Recovery Studies

Level of % Recovery	%Mean Recovery		Standard Deviation		Co-efficient of Variation (%R.S.D.)		Standard Error	
	ACE	SER	ACE	SER	ACE	SER	ACE	SER
80	97.32	98.65	0.847	0.059	0.857	0.058	0.481	0.033
100	99.56	99.43	0.753	0.363	0.755	0.357	0.445	0.212
120	99.04	99.62	0.437	0.153	0.421	0.162	0.263	0.093

Table 4. Regression and Optical Characteristics of Aceclofenac and Serratiopeptidase

Parameter	Aceclofenac	Serratiopeptidase
Working λ	274	279
Beer's Law range	10-50 µg/ml	1.5-7.5 µg/ml
Slope	0.0053	0.0002
Intercept	0.0004	0.0021
Regression coefficient (r^2)	0.986	0.989

Table 5. Data for LOD and LOQ of Aceclofenac and Serratiopeptidase

Drug	LOD (µg/ml)	LOQ (µg/ml)
ACE (274 nm)	2.34452	7.085
SER (279 nm)	12.6044	37.89

2.8. Validation Study

The following method parameters were evaluated in order to validate the quality of the proposed method: linearity, recovery, precision. Linear range of the proposed method was established by analysis of five standard calibration solutions. Recoveries were calculated using the slop of the linear regression. The intra and inter-day precision were evaluated by repeating the assay method one times on the same day and on three consecutive days, respectively.

3. Accuracy and Precision

The accuracy study was performed by addition of known amounts of ACE and SER to known concentration. Precision of the method were assessed by intra and inter-day validation. The intra and inter-day precision were determined by determining the concentrations of ACE and SER in synthetic mixture in five replicates for three different concentration levels. The intra and inter-day precision were obtained by repeating the assay method three times on the same day and on three consecutive days, respectively. The repeatability of the method was expressed as the %RSD. Accuracy was expressed as the percent deviation of the mean determined concentration against the spiked concentration. (Table 3), summarizes the mean values of accuracy and precision for both intra and inter-day assays. Both precision and accuracy results indicated satisfactory precision of the proposed methods according to the FDA guidelines.[15]

3.1. Calibration Curve

The calibration curve showed a good linearity in the concentration range of 10-50 µg/ml and 1.5-7.5 µg/ml with correlation coefficient (r^2>0.986 and 0.989) respectively for Aceclofenac and Serratiopeptidase (Table 4). The Limit of Detection (LOD) and Limit of Quantification (LOQ) (Table 5) values for Aceclofenac and Serratiopeptidase were determined according to ICH[16] recommendations considering the SD of the response and the slope.

Table 6. The standard deviation (S.D.), relative standard deviation (%R.S.D.), and standard error (S.E.) calculated are low, indicating high degree of precision of the method

Drug	%Mean*	S.D*	%R.S.D.*	S.E.*
ACE	98.92	0.916	0.923	0.410
SERA	99.85	0.231	0.231	0.102

4. Procedure and Precision

The standard deviation (S.D.), relative standard deviation (%R.S.D.), and standard error (S.E.) calculated are low, indicating high degree of precision of the method (Table 6).

5. Result and Discussion

The standard deviation (S.D.), relative standard deviation (%R.S.D.), and standard error (S.E.) calculated are low, indicating high degree of precision of the method. The %R.S.D. is less than 2% as required by USP and ICH guidelines.[16]

Proposed method for first derivative estimation of Aceclofenac and Serratiopeptidase in combined sample solutions was found to be simple, accurate and reproducible. Data for validation and precision studies are given in Tables 4, 5 and 6. Once the equations are determined, analysis required only the measuring of the absorbance of the sample solution at the two wavelengths selected, followed by a few simple calculations.

The standard deviation (S.D.), relative standard deviation (%R.S.D.) and standard error (S.E.) calculated are low, indicating high degree of precision of the method. The %R.S.D. is less than 2% as required by USP and ICH guidelines complies in our method.[16]

6. Conclusions

The method was successfully used to estimate the amount of Aceclofenac and Serratiopeptidase in synthetic mixture containing 100 mg and 15 mg. By observing validation parameters, method was found to be specific, accurate, precise, repeatable and reproducible. This method is simple in calculation, hence can be employed for routine analysis of synthetic mixture as well as dissolution testing.

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Conflicts of Interest

The author declares no conflict of interest.

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