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Development and Validation of Analytical Method for Simultaneous Estimation of Diclofenac Sodium and Serratiopeptidase in Bulk and Tablet Dosage Form

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ABSTRACT

Second order derivative spectroscopy method was developed and validated for the simultaneous estimation of Diclofenac sodium (DFS) and Serratiopeptidase (SPD) in bulk and tablet dosage form. Accurate and Précised UV Spectrophotometric method with good sensitivity has been developed for simultaneous estimation of DFS and SPD. The method employs Second order derivative based on the measurement of absorbance of DFS at ZCP 264.20 nm and SPD at ZCP 295.20 nm. The calibration curve was linear in a concentration range of 5-30 μg/ml for DFS and 25-150 μg/ml for SPD. The developed method was validated as per ICH guideline, for its accuracy, precision, LOD, LOQ and the results were found to be satisfactory, thus the method is specific, rapid and simple with good sensitivity for estimation of DFS and SPD in marketed dosage form.

Keywords: Diclofenac sodium, Serratiopeptidase, Second order derivative method, Validation

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INTRODUCTION:

Diclofenac Sodium belongs to the class of Non-steroidal Anti-inflammatory Agents (NSAIAs) whereas Serratiopeptidase belongs to Proteolytic enzyme (protease). Diclofenac Sodium also has Antipyretic and Analgesic action. It is primarily available as the sodium salt. Serratiopeptidase (Serratia E-15 protease, also known as serralysin, serratiapeptase, serratia peptidase, serratio peptidase, or serrapeptidase) is a proteolytic enzyme (protease) produced by enterobacterium *Serratia* sp. E-15. The structures of Diclofenac Sodium and Serratiopeptidase are shown in figure1.[1]

Analytical method development and validation play an important role in the drug discovery, development, and manufacture of pharmaceuticals. The official test methods that result from analytical method development are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products and to ensure compliance with quality and safety standards. [1]
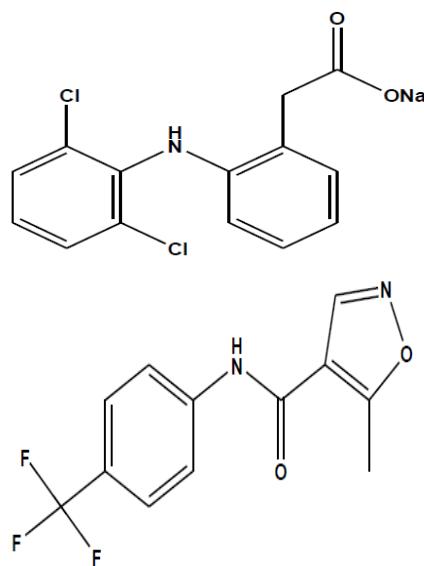
Figure: 1 Structure of Diclofenac Sodium and Serratiopeptidase

Derivative zero crossing Spectrophotometry:

It is a useful means of resolving the overlapping spectra and eliminating the interference. It involves conversion of normal spectrum to first, second or higher order spectras where the amplitude in the derivative spectra is proportional to the concentration of analyte provided the Beers law is obeyed. The successive development of the zero-crossing technique was accordingly due to the feature of derivative spectra to show signals with both positive and negative value. This technique exploits the signal crossing through the abscissa axis, for a given component of a mixture, to assign the absorbance value to remaining components. Zero crossing technique is particularly effective in the analysis of several complex mixtures, when wide peaks overlapping are present in the corresponding zero-order spectrum.

However, suitable analytical signals are often placed on the peak shoulders or characterized by a too low absorbance. This could heavily limit the accuracy and precision of the method, as the low stability of such signals is well known. [2]

Literature review reveals that there are several spectroscopic methods like First order derivative and Absorption ratio methods reported for the estimation of Diclofenac Sodium and Serratiopeptidase. But second order derivative method is not reported for this combination.
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Hence it was thought worthwhile to develop analytical methods for simultaneous determination of Diclofenac Sodium & Serratiopeptidase in their bulk form and tablet dosage form. [3] [4] [5] [6]

MATERIALS AND METHODS

Instrument:

➢ Double beam UV-spectrophotometer (Shimadzu-1800)

➢ UV Probe Version 1.03

➢ Shimadzu electronic balance BL220H

➢ Sonicator : Ultrasonic Cleaner

Materials:

➢ API   Serratiopeptidase (as gift sample procured from OM lab)

➢ API   Diclofenac Sodium

➢ CIPZEN - D tablets (label claim 50 mg Diclo + 10 mg Serratio)

All apparatus and instrument were calibrated and validated as per SOP/protocol before starting the experimental work

Reagents and Chemicals:

Table:1 List of Chemicals and Reagents			
Sr. No.	Chemicals/Reagents	Grade	Company
1	Acetonitrile	HPLC	Merck
2	Methanol	HPLC	Merck
3	Water	HPLC	Merck
4	Ethanol	NA	Merck
5	Potassium dihydrogen phosphate monobasic	HPLC	Spectro chem
6	Acetone	NA	Merck
7	0.1 HCl	NA	NA

Marketed formulation:

CIPZEN - D tablet

Label claim: Diclofenac sodium 50 mg

        Serratiopeptidase10 mg

Identification of Pure Drug

Melting point determination

It was carried out by Capillary melting point method. A few amount of the compound was placed in a thin wall capillary tube 10-15 cm long, about 1 mm in inside diameter, and fused at one end. The capillary containing the sample and a thermometer was suspended in oil bath so that they can be heated slowly and evenly. The temperature range over which the sample was melted, taken as the melting point. And it was compare with standard. [7]

Solubility

Solubility of compound was determined by UV spectroscopy. In this method, saturated solution of compound was prepared in solvent such as water, methanol, acetonitrile and phosphate buffer. Further it was kept for 24 h filtered and the filtrate was used for further analysis and the absorbance was measured at 274nm and 276nm. Further on the basis of absorbance the drug concentration was calculated in mg/ml. [7]

IR identification

Fourier Transform Infrared Spectroscopy was carried out for solid samples to identify the presence of various functional groups present in drug. The samples were prepared by the potassium bromide disc method. Powders (20mg drug in 280mg KBr) were triturated in agate mortar and pestle to produce fine and uniform mixture. The pellets were prepared by compressing the powders at 20 psi for 10 min using Potassium bromide - press. Pure potassium bromide powder was used as background, and for baseline correction. Prepared sample disc was placed in a sample holder and transferred to sample compartment. Samples were scanned in the region of 4000-400 cm-1 using a bruker FTIR spectrometer and it was compared with standard. [7]

Development of UV Second Order Derivative Spectroscopy:

Condition used:

➢ Mode : Spectrum

➢ Scan speed : Medium

➢ Wavelength range : 200-400 nm

➢ Derivative order : 2

➢ Delta lamda : 8 nm

➢ Scaling factor : 10

Selection of solvent for Diclofenac sodium and Serratiopeptidase:

Many solvents like Water, Methanol, 0.1N Sodium Hydroxide (NaOH), and Ethanol were tried for better analysis. Among all, water was selected as the best solvent for UV analysis. The overlain spectra of Diclofenac sodium and Serratiopeptidase were overlapped which showed a feasibility of using this solvent for Spectrophotometric analysis with concern to simultaneous estimation of these drugs.

Selection of analytical wavelength for Diclofenac sodium and Serratiopeptidase:

5-30 µg/ml solutions of Diclofenac sodium were prepared in water and spectrum was recorded between 200-400 nm. Second derivative spectrums for above concentration were taken. Similarly 25-150 µg/ml solutions of Serratiopeptidase were prepared in water and spectrum was recorded between 200-400 nm and Second derivative spectrums were taken. The derivative spectra of Diclofenac sodium and Serratiopeptidase at different concentration were recorded. The zero crossing point (ZCP) of Diclofenac sodium at which Serratiopeptidase is measured and ZCP of Serratiopeptidase at which Diclofenac sodium was measured for the overlain spectra of both.

Preparation of standard stock solution

Diclofenac sodium standard stock solution: (100 µg/ml):

A 10 mg of standard Diclofenac sodium was weighed and transferred to a 100 ml volumetric flask and dissolved in 25 ml water. The flask was shaken and volume was made up to the mark with water to give a solution containing 100µg/ml Diclofenac sodium.

Serratiopeptidase standard stock solution: (500 µg/ml):

A 50 mg of Serratiopeptidase standard was accurately weighed and transferred to a 100 ml volumetric flask and dissolved in 25 ml water. The flask was Sonicated for 10 minutes and volume was made up to the mark with methanol to give a solution containing 500µg/ml Serratiopeptidase.

Calibration curve for Diclofenac sodium and Serratiopeptidase

Calibration curve of Diclofenac sodium (5-30 µg/ml)

Appropriate volume of aliquots from standard Diclofenac sodium stock solutions were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the water to obtain concentration of and 5, 10, 15, 20, 25 and 30 µg/ml. The second derivative (D2) curve of each solution was recorded. D2 absorbance at ZCP of Serratiopeptidase was measured and the plot of D2 absorbance vs. concentration was plotted. The straight-line equation was determined.

Calibration curve of Serratiopeptidase (25-150 µg/ml)

Appropriate volume of aliquots from standard Serratiopeptidase stock solutions was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the water to obtain concentration of 25, 50, 75, 100, 125 and 150 µg/ml. The second derivative (D2) curve of each solution was recorded. D2 absorbance at Diclofenac sodium was measured and the plot of D2 absorbance vs. concentration was plotted. The straight-line equation was determined.

Linearity

The linearity response was determined by analyzing independent levels of concentrations in the range of 5-30 and 25-150 µg/ml for Diclofenac sodium and Serratiopeptidase respectively. Absorbance of each solution was measured at ZCP of Serratiopeptidase and Diclofenac sodium respectively using developed method. Calibration curve of absorbance Vs concentration was plotted and the correlation coefficient, regression line equations for Diclofenac sodium and Serratiopeptidase were determined respectively.

Accuracy

The accuracy studies were carried out by addition of standard drug to the sample at 3 different concentration levels (50, 100 and 150 %) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery of Diclofenac sodium and Serratiopeptidase by standard addition method. Accuracy is the closeness of the test results obtained by the method to the true value.

Precision

1. Repeatability

6 replicates of 20 µg/ml concentrations of Diclofenac sodium and 100 µg/ml of Serratiopeptidase were prepared and absorbance was measured at ZCP of Serratiopeptidase and Diclofenac sodium respectively. SD and %RSD were calculated.

2 Interday precision

Standard solutions containing 10, 20 and 30 µg/ml Diclofenac sodium and 50, 100 and 150µg/ml Serratiopeptidase were analyzed on three different days as per the procedure. The absorbance of solutions was measured at ZCP of Serratiopeptidase and Diclofenac sodium respectively. SD and %RSD were calculated

3 Intraday precision

Standard solutions containing 10, 20 and 30 µg/ml Diclofenac sodium and 50, 100 and 150µg/ml Serratiopeptidase were analyzed 3 times on the same day as per the procedure. The absorbance of solutions was measured at ZCP of Serratiopeptidase and Diclofenac sodium respectively. SD and %RSD were calculated.

Limit of Detection

Calibration curve was carried out for 6 times and the standard deviation (SD) of the intercepts were calculated by the formula as mentioned below:

LOD= (3.3*SD)/Slope

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

Limit of Quantitation

Calibration curve was repeated for 6 times and the standard deviation (SD) of the intercepts was calculated by the formula as specified below:

LOQ= (10*SD)/Slope

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

Robustness

For the determination of robustness 3 different mixture of same concentration of samples were analyzed at different wavelength. The absorbance at 263.2, 264.2 and 265.2 nm and 294.2, 295.2 and 296.2 nm were recorded and %R.S.D. was calculated.

Determination of Diclofenac sodium and Serratiopeptidase in their tablet dosage form (Assay) (Standard addition method)

Sample preparation

For estimation of Diclofenac sodium and Serratiopeptidase, 20 tablets were weighted and powdered with the help of mortar and pestle. A quantity of the powdered tablet equivalent to 50 mg of Diclofenac sodium and 10 mg of Serratiopeptidase was weighed accurately and 240 mg standard Serratiopeptidase powder was added into it (standard addition) and transferred to volumetric flask of 100 ml capacity. 100 ml of water was transferred to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with water. The above solution was filtered through whatman filter paper (0.45µ). From this solution 20 ml was transferred to volumetric flask of 100 ml capacity. Volume was made up to the mark to give a solution containing 100 µg/ml Diclofenac sodium and 500 µg/ml Serratiopeptidase (solution A). From the solution A, 4 ml was transferred to volumetric flask of 100 ml capacity and volume was made up to the mark to give a solution containing 20 µg/ml Diclofenac sodium and 100

µg/ml Serratiopeptidase. This solution was used for the estimation of Diclofenac sodium and Serratiopeptidase.

Estimation of Diclofenac sodium and Serratiopeptidase

The derivative response of above solution was measured at ZCP of Serratiopeptidase for Diclofenac sodium and at ZCP of Diclofenac sodium for quantification of Serratiopeptidase respectively. The amount of Diclofenac sodium and Serratiopeptidase present in the sample solution were determined by fitting derivative responses into the equation of the straight line representing the calibration curves for Diclofenac sodium and Serratiopeptidase.

RESULT AND DISCUSSION

Identification of drugs:

Melting Point:

Table 2: Melting Point of Diclofenac sodium and Serratiopeptidase		
APIs	Observed	Standard
Diclofenac sodium	164-166°C	163-168°C
Serratiopeptidase	284-286°C	283-285°C

Solubility Study:

Diclofenac sodium : Soluble in water, Acetone, Ethanol, Methanol, Acetonitrile

Serratiopeptidase : Soluble in water

Discussion: From the obtained result, Serratiopeptidase was found to be soluble in water. Diclofenac sodium was found to be soluble in water, methanol, Acetonitrile (ACN), and Ethanol. Therefore, single solvent water could be used for estimation of both drugs.

**IR Identification:**

Diclofenac sodium FTIR spectra:
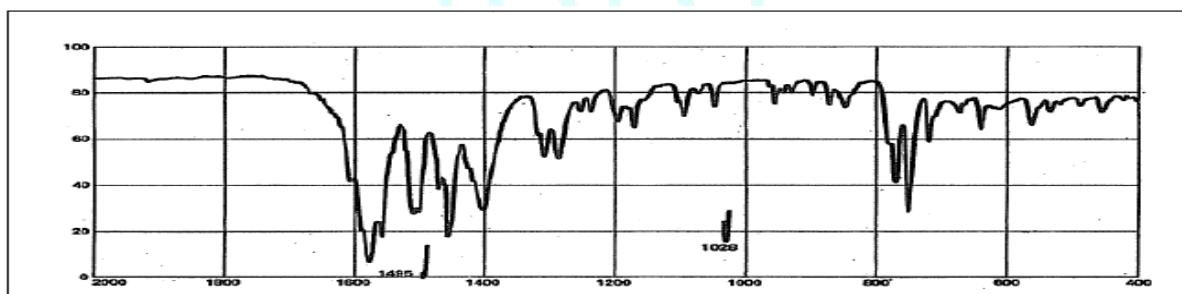
**Figure 2 : FTIR spectrum of Diclofenac sodium (Reference-As per IP 2010)**
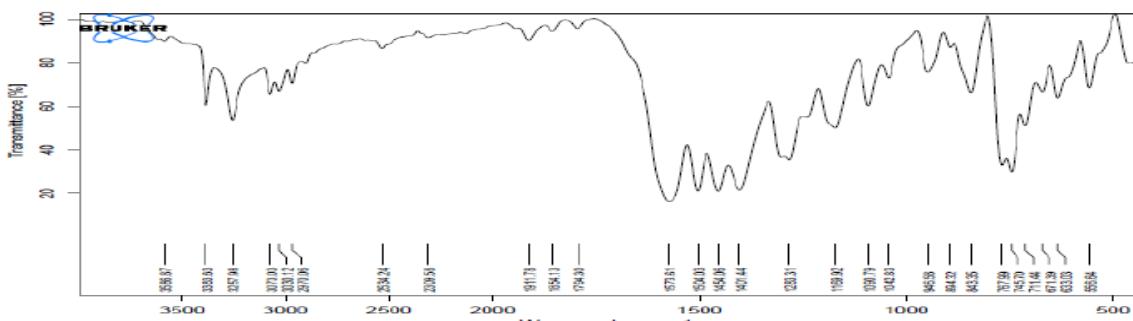
Figure 3: FTIR spectrum of Diclofenac sodium

Table 3: IR interpretation of Diclofenac sodium		
Sr. No.	Frequency(cm-1)	Characteristics
1	3383.63	N-H stretch
2	2970.06	Aliphatic C-H stretching
3	1573.61	C=O stretching of carboxyl ion
4	1504.03	Aromatic ring
5	1283.31	Skeletal C=C vibration
6	1169.92	C-O stretch
7	1090.79	Aromatic C-H in-plane bend
8	767.99	C-Cl

Discussion: IR spectrum of Diclofenac sodium sample was interpreted. From these, we can say that the given sample may be Diclofenac sodium.

Serratiopeptidase:
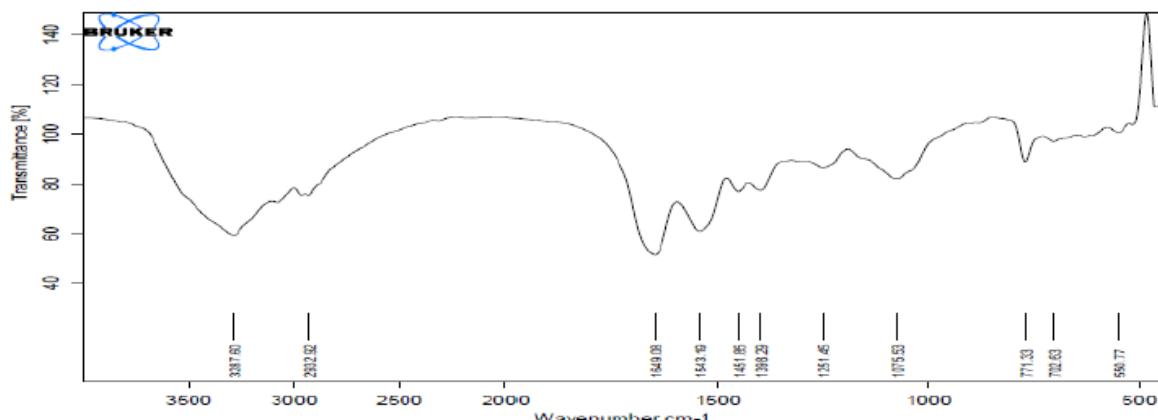
Figure 4: FTIR spectrum of Serratiopeptidase

Table 4: IR interpretation of Serratiopeptidase		
Sr.No	Frequency(cm-1)	Characteristics
1	3287.60	N-H Stretching
2	2932.92	C-H Aliphatic stretching
3	1649.08	C=O Stretching
4	1543.19	Aromatic ring
5	1251.45	C-N Stretching
6	1075.53	C-O Stretching
7	771.33	C-F

Discussion: IR spectrum of Serratiopeptidase sample was interpreted. From these, we can say that the given sample may be Serratiopeptidase.

Method Development by Second Order Derivative UV Spectroscopy:

Selection of solvent for Diclofenac sodium and Serratiopeptidase:

Water was selected as solvent because both the drugs were soluble in it and spectra of Diclofenac sodium and Serratiopeptidase, when overlapped, show feasibility of using this solvent for Spectrophotometric analysis for their estimation by UV method.

Analytical wavelength for Diclofenac sodium and Serratiopeptidase:
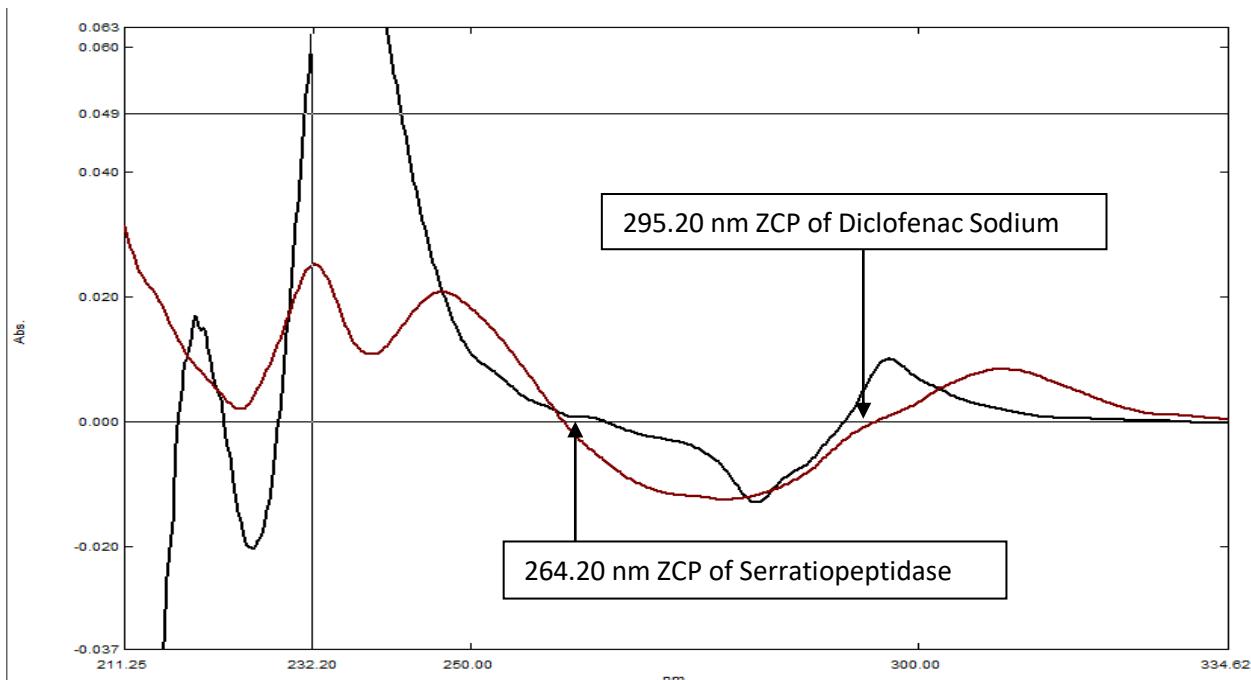
Figure 5: Second order derivative spectrum overlay of Diclofenac sodium (15 µg/ml) and Serratiopeptidase (75 µg/ml)
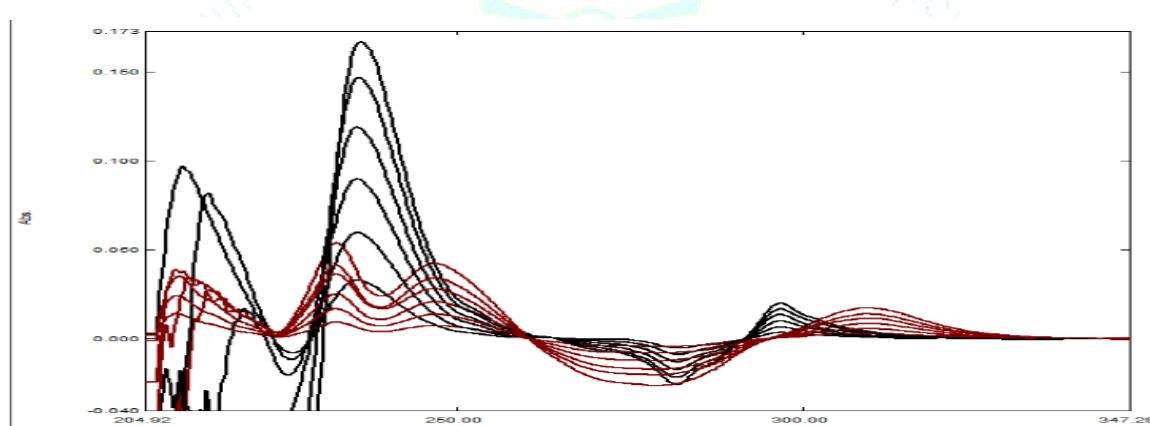
Figure 6: Second order derivative spectrum overlay of Diclofenac sodium (5-30 µg/ml) and Serratiopeptidase (25-150 µg/ml)

Discussion: The zero crossing point (ZCP) of Diclofenac sodium was 295.20 nm where second order derivative of Serratiopeptidase measured and ZCP of Serratiopeptidase was 264.20 nm where second derivative of Diclofenac sodium measured.

Validation of UV Spectrophotometric Method:

Linearity

Calibration curve for the Diclofenac sodium (5-30 µg/ml)

Absorbance at 264.20 nm ZCP of Serratiopeptidase was measured for following concentrations 5, 10, 15, 20, 25 and 30 µg/ml. Absorbances were obtained and the graph of calibration curve was obtained.

Table 5: Calibration curve for Diclofenac sodium	
Conc. (µg/ml)	**Mean Absorbance ± S.D.**
5	0.0019 ± 0.00015
10	0.0036 ± 0.00010
15	0.0054 ± 0.00015
20	0.0072 ± 0.00025
25	0.0088 ± 0.00025
30	0.0108 ± 0.00032
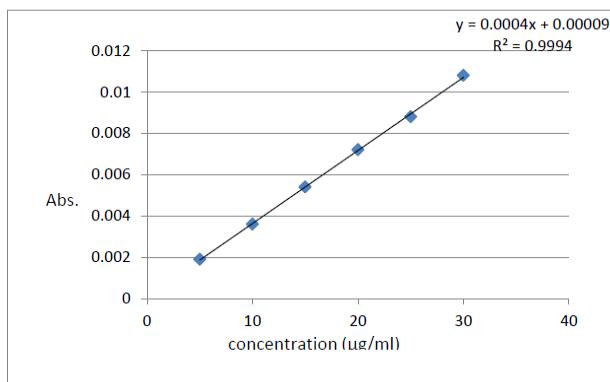
Figure7: Graph of Calibration curve for Diclofenac Sodium

Discussion: Linearity range for Serratiopeptidase was found to be 25-150 μg/ml in. water. Regression Equation for Serratiopeptidase at 295.20 nm: Y= 0.0004x+ 0.00009. R2 value: 0.9989.

Calibration curve for the Serratiopeptidase (25-150 μg/ml)

Table 6: Calibration curve for Serratiopeptidase	
Conc. (μg/ml)	Mean Absorbance ± S.D.
25	0.0028 ± 0.00011
50	0.0055 ± 0.00015
75	0.0084 ± 0.00015
100	0.0116 ± 0.00010
125	0.0140 ± 0.00015
150	0.0168 ± 0.00020

Absorbances at 295.20 nm ZCP of Serratiopeptidase were measured for following concentrations 25, 50,75,100, 125 and 150μg/ml. Absorbances were obtained and the graph of calibration curve was obtained.
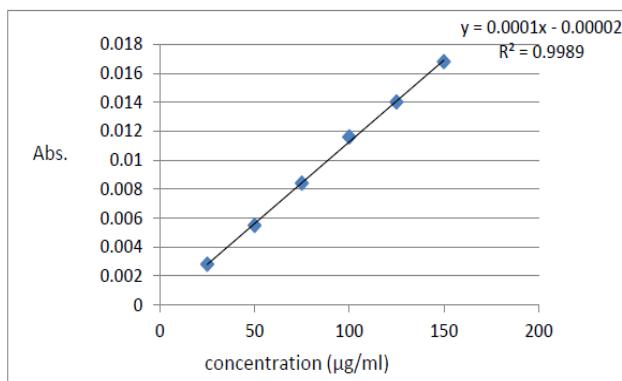
Figure 8: Graph of Calibration curve for Serratiopeptidase

Discussion: Linearity range for Serratiopeptidase was found to be 25-150 μg/ml in. water. Regression Equation for Serratiopeptidase at 295.20 nm: Y= 0.0001x+ 0.00002. R2 value: 0.9989.

Accuracy

Diclofenac sodium

Concentration of Preanalyzed sample of Diclofenac sodium: 10μg/ml

Table 7: Accuracy data of Diclofenac sodium							
Level	Amt of added std (μg/ml)	Total conc. (μg/ml)	Abs	Avg. Abs.	Conc. found (μg/ml)	% recovery	Mean ± SD
50%	5	15	0.0059	0.00606	14.94	99.60	100.39 ± 0.745
	5	15	0.0061				
	5	15	0.0062				
100%	10	20	0.0081	0.00813	20.10	100.50	
	10	20	0.0080				
	10	20	0.0083				
150%	15	25	0.0101	0.0102	25.27	101.08	
	15	25	0.0103				
	15	25	0.0102				

Discussion: Result obtained reveals that % recovery of Diclofenac sodium was within acceptance criteria given in ICH i.e. 98-102%.

Serratiopeptidase

Concentration of Preanalyzed sample of Serratiopeptidase: 50 μg/ml

Table 8 : Accuracy data of Serratiopeptidase						
Level	Amt of added std (μg/ml)	Total conc. (μg/ml)	Abs	Avg. Abs.	Conc. found (μg/ml)	% recovery	Mean ± SD
50%	25	75	0.0073	0.0074	25.11	98.93	99.38± 0.465
	25	75	0.0074				
	25	75	0.0075				
100%	50	100	0.0100	0.00996	99.67	99.86	
	50	100	0.0099				
	50	100	0.0100				
150%	75	125	0.0123	0.0124	124.20	99.36	
	75	125	0.0125				
	75	125	0.0124				

Discussion: Result obtained reveals that % recovery of Serratiopeptidase was within acceptance criteria given in ICH i.e. 98-102%.

Precision

Repeatability

Table 9: Repeatability data of Diclofenac sodium and Serratiopeptidase						
Standard drug	Target conc. (μg/ml)	Abs of sample	Conc. found (μg/ml)	Mean	SD	%RSD
Diclofenac sodium	20	0.0079	19.525	19.48	0.188	0.965
	20	0.0079	19.525			
	20	0.0080	19.775			
	20	0.0078	19.225			
	20	0.0078	19.225			
	20	0.0079	19.525			
Serratiopeptidase	100	0.0101	101.2	100.03	0.752	0.752
	100	0.0100	100.2			
	100	0.0100	100.2			
	100	0.0099	99.2			
	100	0.0099	99.2			
	100	0.0100	100.2			

Discussion: The % RSD for Repeatability of both the drugs was found to be less than 2. So, it was concluded that proposed method for estimation of Diclofenac sodium and Serratiopeptidase is précised in nature.

Interday precision:

Conc. (µg/ml)	Absorbance			Conc. found (µg/ml)			Mean ± S.D.	%RSD
	1	**2**	**3**	**1**	**2**	**3**		
10	0.0041	0.0042	0.0041	10.025	10.275	10.025	10.108±0.144	1.42
20	0.0079	0.0078	0.0076	19.525	19.275	18.775	19.131±0.381	1.98
30	0.0119	0.0118	0.0121	29.525	29.275	30.225	29.608±0.381	1.28

Table 10: Interday precision data for Diclofenac sodium

Conc. (µg/ml)	Absorbance			Conc. found (µg/ml)			Mean ± S.D.	%RSD
	1	**2**	**3**	**1**	**2**	**3**		
50	0.0050	0.0049	0.0049	50.7	49.7	49.7	49.53±0.577	1.16
100	0.0100	0.0101	0.0101	100.7	103.7	101.7	101.53±1.527	1.50
150	0.0147	0.0148	0.0150	147.7	148.7	150.7	148.53±1.527	1.02

Table 11: Interday precision data for Serratiopeptidase

Discussion*:* The % RSD for Interday precision of both the drugs was found to be less than 2. So, it was concluded that proposed method for estimation of Diclofenac sodium and Serratiopeptidase is précised in nature.

Intraday precision

Conc. (µg/ml)	Absorbance			Conc. found (µg/ml)			Mean ± S.D.	%RSD
	1	**2**	**3**	**1**	**2**	**3**		
10	0.0041	0.0042	0.0041	10.025	10.275	10.025	10.108±0.144	1.42
20	0.0079	0.0081	0.0078	19.525	20.025	19.275	19.608±0.381	1.94
30	0.0119	0.0120	0.0117	29.525	29.775	29.025	29.441±0.381	1.29

Table 12: Intraday precision data for Diclofenac sodium

Conc. (µg/ml)	Absorbance			Conc. found (µg/ml)			Mean ± S.D.	%RSD
	1	**2**	**3**	**1**	**2**	**3**		
50	0.0050	0.0049	0.0050	50.7	49.7	50.7	49.86±0.5773	1.15
100	0.0101	0.0100	0.0103	101.7	100.7	103.7	101.53±1.527	1.50
150	0.0147	0.0146	0.0149	147.7	146.7	149.7	147.53±1.527	1.03

Table 13: Intraday precision data for Serratiopeptidase

Discussion*:* The % RSD Intraday precision and Inter day precision for of both the drugs was found to be less than 2. So, it was concluded that proposed method for estimation of Diclofenac sodium and Serratiopeptidase is précised in nature.

Limit of Detection

Parameter	Diclofenac sodium	Serratiopeptidase
Slope (n=6)	0.0004	0.0001
SD (n=6)	0.00008	0.00004
LOD (µg/ml)	0.6771	1.3364

Table 14: LOD data Diclofenac sodium and Serratiopeptidase

Discussion*:* The proposed method can detect Diclofenac sodium and Serratiopeptidase at very low level .So, it was concluded that the proposed method is very sensitive in nature.

Limit of Quantitation

LOQ data for Diclofenac sodium and Serratiopeptidase

Table 15: LOQ data Diclofenac sodium and Serratiopeptidase		
Parameter	Diclofenac sodium	Serratiopeptidase
Slope (n=6)	0.0004	0.0001
SD (n=6)	0.00008	0.00004
LOQ (µg/ml)	2.0519	4.0498

Discussion: The proposed method can quantify small amount of drugs with precisely. So, it was concluded that the proposed method is very sensitive in nature.

Robustness

Table 16: Robustness data for Diclofenac sodium						
Conc. (µg/ml)	Abs. at 263.2	Conc. 1	Abs. at 264.2	Conc. 2	Abs. at 265.2	Conc. 3
20	0.0063	15.52	0.0079	19.52	0.0094	23.27
20	0.0064	15.77	0.0078	19.27	0.0093	23.05
20	0.0063	15.52	0.0081	20.02	0.0096	23.77
	Mean	15.60		19.60		23.25
	SD	0.144		0.381		0.38
	%RSD	0.92		1.94		1.63

Table 17: Robustness data for Serratiopeptidase						
Conc. (µg/ml)	Abs. at 294.2	Conc. 1	Abs. at 295.2	Conc. 2	Abs. at 296.2	Conc. 3
100	0.0068	68.7	0.0101	101.7	0.0118	118.7
100	0.0069	69.7	0.0098	98.7	0.0119	119.7
100	0.0069	69.7	0.0100	100.7	0.0121	121.7
	Mean	69.36		99.86		119.53
	SD	0.577		1.527		1.527
	%RSD	0.83		1.52		1.27

Discussion: The % RSD of Diclofenac sodium and Serratiopeptidase in Robustness study was found to be less than 2 which is within the acceptance criteria.

Derivative spectrum of Diclofenac sodium and Serratiopeptidase in tablet dosage formulation (Assay) (Standard Addition Method)

Table 18: % Assay of Diclofenac sodium and Serratiopeptidase(n=6)					
Drug	Conc. in formulation mg/tablet	Conc. taken for assay (µg/ml)	Abs. of sample	Conc. Found (µg/ml)	%Assay ± S.D.
Diclofenac sodium	50 mg	20	0.0081	20.025	100.12 ± 0.223
Serratiopepti dase	10 mg	100	0.0100	100.86	100.86 ± 1.722

Discussion: % Assay of Diclofenac sodium and Serratiopeptidase was found in an acceptance limit so this method could be used for analysis of this combination.

CONCLUSION

The present work involves the development and validation of a simple, accurate and precise Second order derivative UV spectrophotometric method for the assay of Diclofenac sodium and Serratiopeptidase was carried out in Tablet dosage forms.

The wavelengths selected for quantitation were 264.2 nm for (zero crossing point for Diclofenac sodium) and 295.2 nm for (zero crossing point for serratiopeptidase). The method was validated for linearity, precision, accuracy and all the parameters were in line with the standard limits as per laid by the pharmacopoeia. The marketed preparation of this combination was inline with the sample tested.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

Abs: Absorbance; **Avg:** Average**; ACN:** Acetonitrile**; API:** Active pharmaceutical ingredient; USP: United States Pharmacopoeia; **IP:** Indian Pharmacopoeia; BP: British Pharmacopoeia; **UV:** Ultraviolet; **Conc.:** Concentration**; gm:** Gram**; mg:** Milligram**; µg:** Microgram; **ICH:** International Conference on Harmonization **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **SD:** Standard Deviation**; RSD:** Relative Standard Deviation

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