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Simultaneous estimation of Aceclofenac and Serratiopeptidase in Tablet Dosage Form by Absorbance Ratio Method using Visible Spectrophotometry

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ABSTRACT

A UV- visible Spectrophotometric method has been developed and validated for Simultaneous estimation of Aceclofenac and Serratiopeptidase in tablet dosage form using double beam UV Spectrophotometer of thermo Electron Corporation (Heλlos α) with (Ethanol + Water) as a solvent. Absorption maxima of Aceclofenac and Serratiopeptidase in Ethanol diluted with water was found to be 316 nm and 375 nm, respectively. Beer's law was obeyed in the concentration range 30-70 µg/ml for Aceclofenac and 100-300 µg/ml Serratiopeptidase. The mean recoveries obtained for Aceclofenac and Serratiopeptidase were 99.193 % and 99.153 % respectively. The LOD and LOQ for Aceclofenac were found to be 2.334µg/ml and 7.074µg/ml and for Serratiopeptidase 12.50µg/ml and 37.88µg/ml respectively. The methods allow rapid analysis of binary pharmaceutical formulation with accuracy, precision. The method was found to be simple, accurate, precise, economical and robust.

Key words: Aceclofenac, Simultaneous Estimation, Q-analysis, Serratiopeptidase and Isoabsorptive Point.

INTRODUCTION

Aceclofenac (ACE) and Serratiopeptidase (SER) are available in tablet dosage form in the ratio of 100:15. Aceclofenac [1,2,3] is cox-2 inhibitor, chemically [(2, 6-dichloro phenyl) amino] phenyl acetoxy acetic acid used as analgesic & anti inflammatory agent. 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 in presence of Diclofenac.[4,5] Other 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 'proteolytic' 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.

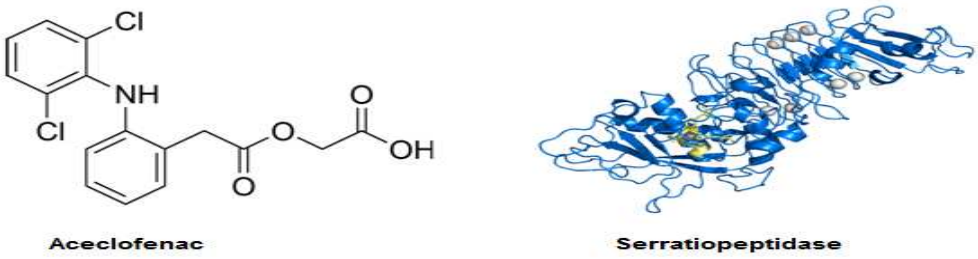

Fig. 1. Structures of Aceclofenac and Serratiopeptidase

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.

MATERIALS AND METHODS

Chemicals and Reagents

Standard gift samples of ACE and SER were obtained from Indica Laboratories, Ahmedabad. Ethanol AR grade was used as a solvent in the study. Double distilled water was used for this study.

Equipment

A double beam UV Spectrophotometer of thermo Electron Corporation (Heλlos α) with computer operated software Vision pro was employed with spectral bandwidth of 2 nm and a pair of 1 cm quartz cell. ACCULAB Sartorius Group ALC-310.3 analytical balance (Gottingen, Germany) and ultra sonic cleaner (Frontline FS 4, Mumbai, India) were used during the study.

Sample preparation

Standard stock solution containing Aceclofenac (ACE) and Serratiopeptidase (SER) was prepared by dissolving 10 mg of Aceclofenac separately in 10 ml Ethanol and 30 mg of Serratiopeptidase in 10 ml distilled water for dissolution of SER, then add 10 ml Ethanol to SER solution and then final volume of both the solution was made up to 100 ml with double distilled water to get the stock solution containing 100 µg/ml of Aceclofenac and 300 µg/ml of Serratiopeptidase in two different 100 ml volumetric flask.

Procedure for determining the wavelength for Q-Analysis and selection of wavelength

5 ml of stock solution of ACE and 6.66 ml of SER from stock solution ware pipette out in 10 ml volumetric flask, diluted up to mark with double distilled water, mixed and 1 ml of Biuret reagent was added, allowed keeping for 5 minute. Solution containing 50 µg/ml of Aceclofenac and 200 µg/ml of Serratiopeptidase were scanned separately in the range of 200-600 nm to determine the wavelength of maximum absorbance for both the drugs. Aceclofenac showed absorbance maxima at 375 nm. The isoabsorptive point was obtained at 316 nm. Overlain spectra for both the drug and selected wavelengths are shown in fig. 2.

Linearity Study

By taking appropriate aliquots of standard solution of ACE (3, 4, 5, 6, 7 ml from 100 ppm stock solution) and SER (3.33, 5, 6.66, 8.33, 10 ml from 300 ppm stock solution) were prepared into 10 ml volumetric flask. The volume was adjusted to mark with double distilled water and mixed. Then 1 ml of Biuret reagent was added to each volumetric flask, kept for 10 min. The blank solution was prepared by adding 10 ml ethanol to 100 ml volumetric flask and make up the volume with double distilled water. Then 10 ml of solution was taken to 10 ml volumetric flask and 1 ml of Biuret reagent was added to it and is used as a blank. Absorbances were taken.

Derivation of Equations

The ratio of two absorbance determined on the two solutions at two different wavelengths is constant. This constant is termed as Q value. The Q value is independent of concentration and thickness of solution and therefore is used to

access the purity of compounds. The absorbance ratio method is a modification of the simultaneous equation procedure. In the quantitative assay of ACE and SER in an admixture by absorbance ratio method, absorbances were measured at any two wavelengths, one is being isoabsorptive point (λ1) and the other is being λmax of one of the component i.e. ACE (λ2) by following equations:

$$Cx = \frac{Qm - Qy}{Qx - Qy} \times \frac{A_1}{ax1}$$

where,

$$Qm = A_2 / A_1$$

and

$$Cy = \frac{Qm - Qx}{Qy - Qx} \times \frac{A_1}{ay1}$$

$$Qx = ax2/ax1$$

$$Qy = ay2/ay1$$

Where, Cx = Concentration of Aceclofenac in ppm
Cy = Concentration of Serratiopeptidase in ppm
A_1 = Absorbance of sample solution at 316 nm
A_2 = Absorbance of sample solution at 375 nm
ax_1 = Absorptivity of standard ACE at 316 nm
ax_2 = Absorptivity of standard ACE at 375 nm
ay_1 = Absorptivity of standard SER at 316 nm
ay_2 = Absorptivity of standard SER at 375 nm

Analysis of marketed formulation

For sample solution, 20 tablets were weighed; their mean weight was determined, and grounded into fine powder in a mortar. An amount of powdered mass equivalent to 5 mg ACE and 0.75 mg SER was accurately weighed, into it 19.25 mg of SER was added to get final stock solution containing 50 ppm of ACE and 200 ppm of SER and transferred in to a 100 ml volumetric flask, 10 ml ethanol was added, the volume was diluted to mark and mixed well, sonicated for 10 min. 10 ml of sample solution was pipette out, transferred it into 10 ml volumetric flask and added 1 ml of Biuret reagent. Allowed it to keep for 5 min and analyzed it.

RESULTS AND DISCUSSION

The proposed method for Q Absorbance Ratio of Aceclofenac and Serratiopeptidase is very simple method and can be employed for routine analysis of Aceclofenac and Serratiopeptidase. The method utilizes 316 nm and 375 nm as analytical wavelengths for estimation of Aceclofenac and Serratiopeptidase.

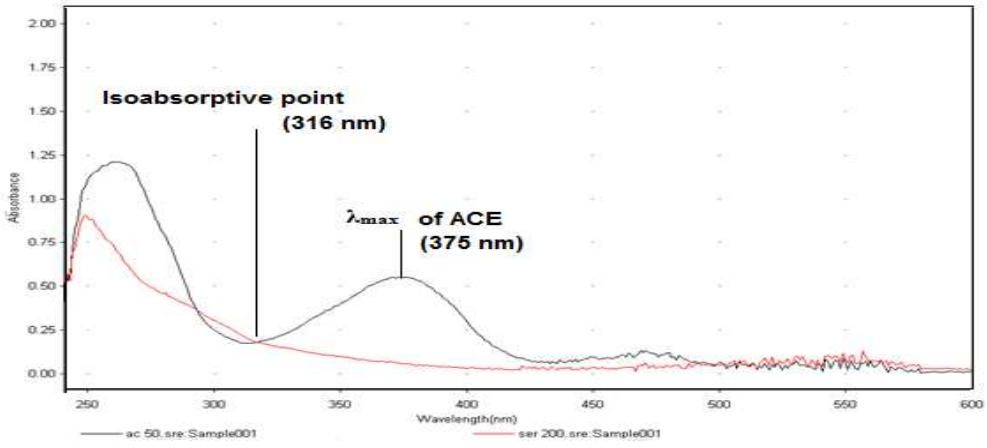

Fig. 2 Overlain spectra of Aceclofenac (50 mcg/ml) and Serratiopeptidase (200 mcg/ml) and isoabsorptive point obtained was 316 nm

The overlain graphs of linearity for ACE and SER are given in fig. 3 and 5 and linearity study graph is given in fig. 4 and 6. R^2 value for ACE were found to be 0.9961 and 0.9968 for at 316 and 375 nm respectively while it were 0.9976 and 0.9955 for SER at 316 and 375 nm respectively. The accuracy of the method was determined by investigating the recovery of the two drugs using spiked concentrations of the two drugs. The result indicated excellent recoveries for the two drugs. Precision for tablet analysis was determined by analysis of tablets containing Aceclofenac and Serratiopeptidase. Results of tablets analysis indicated that there was no interference of the common excipients used in the formulation. The linearity, precision, LOD, LOQ data are give in table 1 and recovery study data, assay result data for both drug are given in table 2 and 3 respectively.

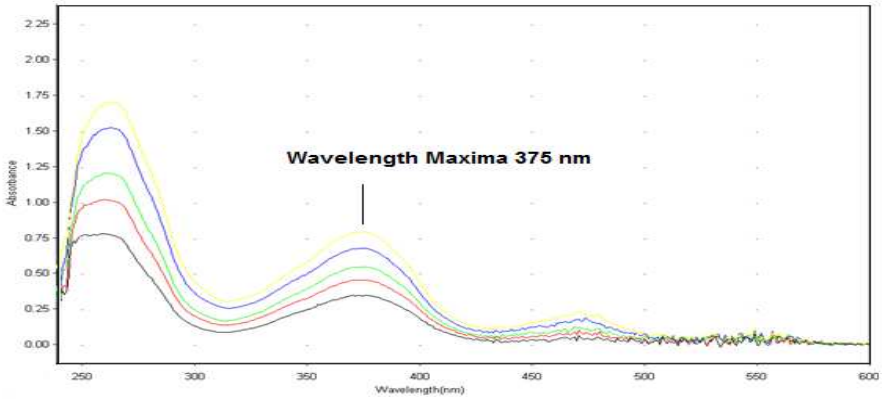

Fig. 3. Overlain spectra of Aceclofenac (30 – 70 mcg/ml)

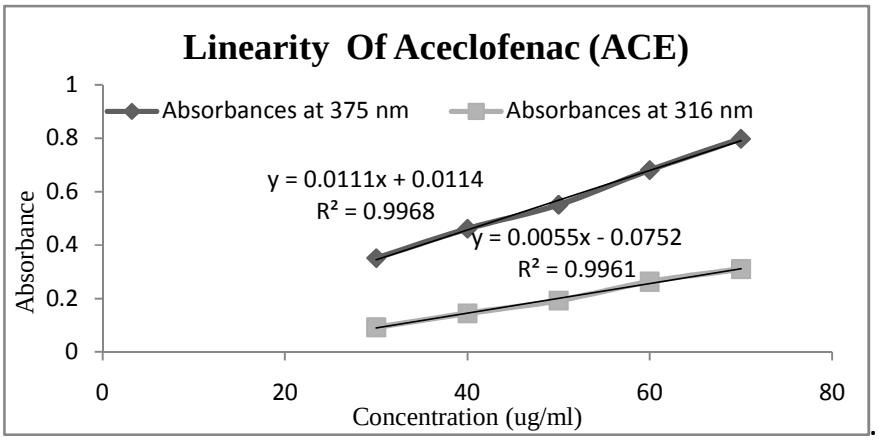

Fig. 4. Linerity study of Aceclofenac (30 – 70 mcg/ml)

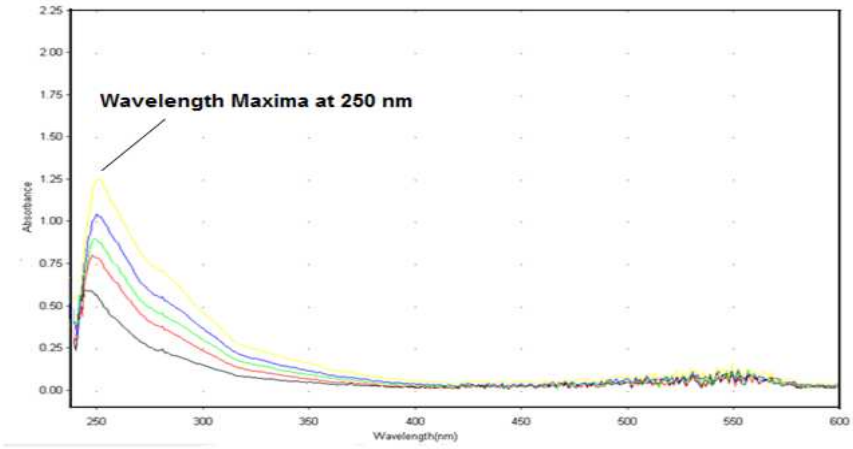

Fig. 5. Overlain spectra of Serratiopeptidase (100 – 300 mcg/ml)

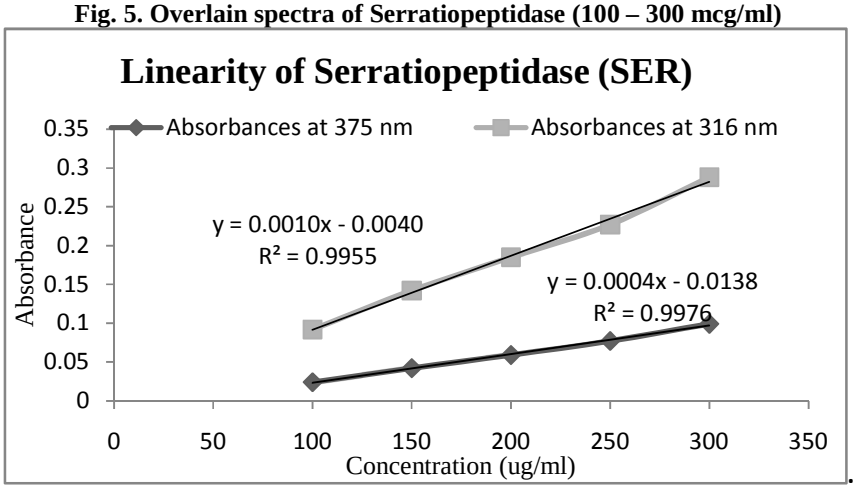

Fig. 6. Linerity study of Serratiopeptidase (100 -300 mcg/ml)

Table 1. Summary of validation parameters for proposed method

Parameters	ACE	SER
Wavelength Range (nm)	200-600	200-600
Beer's law limit (µg/ml)	30-70	100-300
Regression equation (Y = mx + c) at 316 nm	Y = 0.005x - 0.075	Y = 0.001x - 0.004
Slope (m)	0.005	0.001
Intercept (c)	0.075	0.004
Correlation Coefficient (R^2)	R^2 = 0.9968	R^2 = 0.9955
Regression equation (Y = mx + c) at 375 nm	Y = 0.011x - 0.011	Y = 0.000x - 0.013
Slope (m)	0.011	0
Intercept (c)	0.011	0.013
Correlation Coefficient (R^2)	R^2 = 0.9961	R^2 = 0.9976
LOD (µg/ml)	2.33442	12.5004
LOQ (µg/ml)	7.074	37.88
Repeatablity (RSD, n = 6)	1.886084	0.004304
Precision (RSD) %		
Intraday (n = 6)	1.819377%	0.430418%
Interday (n = 6)	0.351%	1.583%
Assay ± SD	98.325 ± 0.18275	100.1318 ± 0.20336

RSD = Relative standard deviation

Table 2. Recovery study of ACE and SER

Drug	Amount Present in Tablet	Amount Added (%)	% Recovery ± SD	Average % recovery
ACE	100	80	99.89 ± 0.85	99.193 ± 0.95
	100	100	98.95 ± 1.07	
	100	120	98.74 ± 0.93	
SER	15	80	98.63 ± 0.68	99.153 ± 0.406
	15	100	99.68 ± 0.39	
	15	120	99.15 ± 0.87	

Table 3. Assay result of ACE and SER

Sample No.	Label Claim		Amount found		% Label Claim	
	ACE (mg/tab)	SER (mg/tab)	ACE (mg/tab)	SER (mg/tab)	ACE (mg/tab)	SER (mg/tab)
1	100	15	99.05	14.53	99.05	96.86
2	100	15	98.97	14.39	98.97	95.93
3	100	15	99	14.82	99	98.8
4	100	15	99.23	14.55	99.23	97

CONCLUSION

The proposed method was validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed method is low, indicating high degree of precision of the method. The result of the recovery study performed show the high degree of accuracy of the proposed method. Hence, this method can be employed successfully for the estimation of Aceclofenac and Serratiopeptidase in routine analysis.

Acknowledgement

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REFERENCES

[1] Merck Index, Merck Research Laboratories, division of Merck and company NJ, USA, 14th ed, **2006**, 5, pp 585-586.
[2] Indian pharmacopoeia, Govt. of India, Ministry of Health and Family Welfare, Delhi: Publication by Controller of Publication; Vol.2 **2007,** pp 681-682.
[3] British Pharmacopoeia, Her Majesty Stationary Office, Vol.1, London, **2009**, pp 44-45
[4] The British Pharmacopoeia, Her Majesty Stationary Office, **1998,** pp 33, 34.
[5] The European Pharmacopoeia, supplement **2000,** 329, 330.
[6] Kousy NM, *J. Pharm. Biomed. Anal.* **, 1999**, 20, 185-194.
[7] Suk LH, Kyun JC, Jin CS, Beom KS, Hyun LM, Geon K. Hwan SD, *J. Pharm. Biomed. Anal.*, **2000**, 23, 775-781.
[8] Vivek SR, Santosh VG, Upasana PP, Mahima RS, *Eurasian J. Anal. Chem.*, **2009**, 4 (2), 184-190.
[9] Yadav AH, Kothapalli LP, Barhate AN, Pawar HI, Pawar CR. *Int. J. Pharm. Research and Development*, **2009**, 1 (10), 1-7.
[10] Uttam DP, Abhijit VN, Aruna VS, Tirumal AD, Kiran VM, *E- J. of Chem.*, **2009**, 6 (1), 289-294.
[11] Rohit S, Chandrakant M, Shital KP, Dhanya KC, Nilofar N, *Research J. Pharm. And Tech.*, **2008**, 1 (4), 430-432.
[12] Golhar MK, Joshi RR, Gupta KR, Wadodkar SG, *Int. J. Chem. Tech. Res.*, **2011**, 3 (2), 786-790.
[13] Sharma SK and Goyal N., *Der Pharmacia Sinica*, **2012**, 3 (1), 5-10.
[14] Hapse SA, Thorave RR, Kadaskar PT, Shirsatrc AS, Dokhe MD, *J. of Pharm. Res.*, **2001**, 4 (11), 3928-3929.
[15] Gandhi SV, Barhate NS, Patel BR, Panchal DD, Bothara KG, *Acta Chromatographica*, **2008**; 20 (2), 175-182.
[16] Joshi R., Pawar N., Katiyar S., Zope D. and Shinde A., *Der Pharmacia Sinica*, **2011**, 2 (3), 164-171.
[17] Singhvi I**,** Goyal A, *Indian J. of Pharm. Sci*, **2007**, 69 (1), 164-165.
[18] Sawant RL, Raskar MA, Ahmed R and Pawar S., *Der Pharma Chemica***, 2012,** 4 (2), 633-638.
[19] Adhikari I., Moitra S., Murthy P. and Mishra U., *Der Pharmacia Sinica***, 2012,** 3 (2), 170-176.
[20] Samina A. Jamadar;et.al; *Der Pharma Chemica*, **2011**, 3 (4), 338-343.