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# UV VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF SERRATIOPEPTIDASE AND DICLOFENAC SODIUM IN THEIR BULK AND COMBINED DOSAGE FORM

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**ABSTRACT:** A simple, rapid, specific, precise and accurate UV Spectrophotometric method for simultaneous estimation of Serratiopeptidase and Diclofenac sodium in bulk and tablet formulation has been developed. UV Spectrophotometric method was developed for the simultaneous estimation of Serratiopeptidase and Diclofenac sodium in tablet formulation. In present work, simple, sensitive, accurate and economical spectroscopic method has been developed for the estimation of Serratiopeptidase and Diclofenac sodium in bulk and its pharmaceutical dosage forms. An absorption maximum was found to be at 275nm for Diclofenac sodium and 264nm for serratipeptidase with the solvent Ethanol 99.99%. Diclofenac sodium follows beer's law in the range of 5-50µg/ml with correlation coefficient of 0.998 and Serratiopeptidase in the range of 5-50µg/ml with correlation coefficient of 0.995. Results of the analysis were validated for accuracy, precision, LOD, LOQ and were found to be satisfactory. The proposed method is simple, rapid and suitable for the routine quality control analysis.

**INTRODUCTION:** Serratiopeptidase [1-4] is a proteolytic enzyme (protease) produced by enterobacterium Serratia sp. E-15. This microorganism was originally isolated in the late 1960s from silkworm Bombyx mori L. (intestine). Serratiopeptidase is present in the silkworm intestine and allows the emerging moth to dissolve its cocoon. Serratiopeptidase is produced by purification from culture of Serratia E 15 bacteria. Today, Serratiopeptidase is produced by fermentation technology. Serratiopeptidase is primarily indicated in the condition of haematoma, inflammation, oedema osteoarthritis, thrombosis.

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**Diclofenac Sodium** [1-2] is sodium 2-[(2,6-dichlorophenyl)- amino] phenylacetate. It is a synthetic non-steroidal anti-inflammatory agent with analgesic, anti-inflammatory and antipyretic activity. Its mechanism of action is associated principally with the inhibition of prostaglandin synthesis (specifically, inhibition of cyclooxygenase).

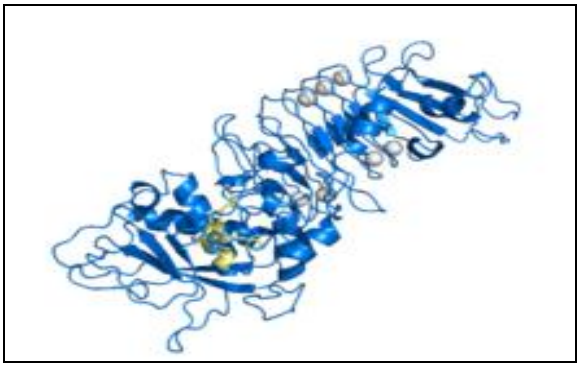

**FIG.1: CRYSTAL STRUCTURE OF SERRALYSIN WITH CO-ORDINATED ZINC (GREY) AND CALCIUM (WHITE)**

Diclofenac sodium is a non-steroidal anti-inflammatory drug taken to reduce inflammation and as an analgesic reducing pain in conditions such as arthritis or acute injury. It can also be used to reduce menstrual pain, dysmenorrhea.

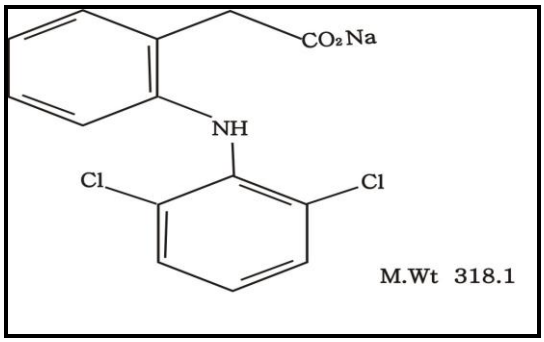


**FIG.2: STRUCTURE OF DICLOFENAC SODIUM**

## MATERIALS AND METHODS:

1. Diclofenac sodium (Zydus cadila, PTC Thane)
2. Serratiopeptidase (Analab fine chemicals)
3. Marketed formulation : Emanzen-D (EMCURE Pharmaceuticals Pvt.Ltd)
4. Ethanol: 99.99%

**Instruments:**

1. **UV Spectrophotometer Model:** Shimadzu UV-1700
2. **Analytical balance :** Shimadzu, AX 200
3. **Sonicator :** Dakshin (ultrasonicator)
4. 

**Method Development:**

**A. Preparation of Standard Stock Solutions**:
Stock solutions (100µg/ml) of Diclofenac sodium and Serratiopeptidase were prepared by dissolving accurately 10 mg of each drug in 100 ml (99.99% ethanol).

**B. Preparation of Tablet stock solution:**
Tablet stock solution was prepared by triturating a tablet to a fine powder, then transfer it into the 100ml volumetric flask and dissolving in 100ml of (99.99% ethanol).

**C. Preparation of working standard solutions**:
Working standard solutions were prepared by pipetting out 1ml SER and 5ml DC from stock solutions in 10 ml volumetric flask and diluting it with distilled water up to the mark.

**D. Preparation of bulk mixture**:
Bulk mixtures were prepared by pipetting out 1ml SER and 5ml DC from stock solutions in 10 ml volumetric flask and diluting it with distilled water up to the mark.

**E. Preparation of Tablet mixture**:
Tablet mixtures were prepared by pipetting out 1ml from stock solution in 10 ml volumetric flask and diluted it with distilled water upto the mark.

**F. Selection of Analytical Wavelengths**:
Appropriate dilutions with distilled water were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. For DC, 275 nm was selected as the working wavelength and SER, show absorbance maxima at 264 nm respectively.

**Preparation of calibration curve:**
From the standard stock solution fresh aliquots were pipetted out and suitably diluted with distilled water to get final concentration in the range of 5-50µg/ml. The solutions were scanned under spectrum mode for 200-400nm wavelength range and a sharp peak was obtained at 275nm for Diclofenac sodium and 264nm for Serratiopeptidase.

A calibration curve was plotted taking an absorbance on y-axis against concentration of standard solution on x-axis (**Fig 1**) and (**Fig 2**) The method was applied for known sample solution and was found to be satisfactory for the analysis of tablet dosage forms.

**Optical characteristics:**
The optical characteristics such as beer's law limit, % RSD were calculated. Regression characteristics like slope intercept, correlation coefficient, LOD, LOQ, standard deviation were calculated **(Table1).**

**Analysis of Mixed Standards**:
The validity of the formed equations was checked by preparing mixed standards, measuring their absorbances at the respective wavelengths and calculating their concentration using the prepared simultaneous equations. Mixed standards in the Beer- Lambert's range for each drug in the ratio of 1:5 were prepared by diluting appropriate volumes from the standard stock solutions with distilled water. **(Table 2).**

**Analysis of Tablet Formulations:** Marketed tablets containing 10 mg of SER and 50 mg of DC were used. An accurately weighed quantity of tablet powder equivalent to 10 mg of SER and 50 mg of DC were transferred to 100 ml volumetric flask and dissolved in 25 ml of (99.99%) ethanol by sonicating for 10 min and volume was then adjusted up to 100 ml with same solvent. The solution was filtered through Whatmann filter paper No. 41 and aliquot portion of filtrate was diluted to obtain solution in the ratio 1:5. The absorbance of sample solution was measured at selected wavelengths. **(Table 3)**

**Validation Parameters:** [5]

**Methods:**

The developed four methods were validated for following parameters.

1) **Precision:**

**I) Repeatability:**

**A. Preparation of bulk mixture**: **(Table4)**

Bulk mixtures were prepared by pipetting out 1ml SER and 5ml DC from stock solutions in 10 ml volumetric flask and diluting it with distilled water up to the mark.

**B. Preparation of Tablet mixture**: **(Table5)**

Tablet mixtures were prepared by pipetting out 1ml from stock solution in 10 ml volumetric flask and diluted it with distilled water upto the mark.

**II) Intermediate precision (Inter-day and Intra-day precision): (Table no.6)**

**A. Preparation of bulk mixture**:

Bulk mixtures were prepared by pipetting out 1ml SER and 5ml DC from stock solutions in 10 ml volumetric flask and diluting it with distilled water up to the mark.

**B. Preparation of Tablet mixture**:

Tablet mixtures were prepared by pipetting out 1ml from stock solution in 10 ml volumetric flask and diluted it with distilled water upto the mark.

**Accuracy: (Table 7)**

Recovery studies were carried out by pipetting out 1ml from tablet stock solution in 10 ml volumetric flask and in that 0.8ml (80%), 1.0ml (100%) and 1.1ml (120%) bulk mixtures of DC and SER were added and absorbance were measured at selected maximum wavelength.

**Linearity and range: (Table 8)** Stock solutions (100µg/ml) of Diclofenac sodium and Serratiopeptidase were prepared by dissolving accurately 10 mg of each drug in (99.99%) 100 ml ethanol then by preparing different concentrations of dilutions (5-50µg/ml) with distilled water and absorbance were measured.

**Simultaneous Equation Method:**

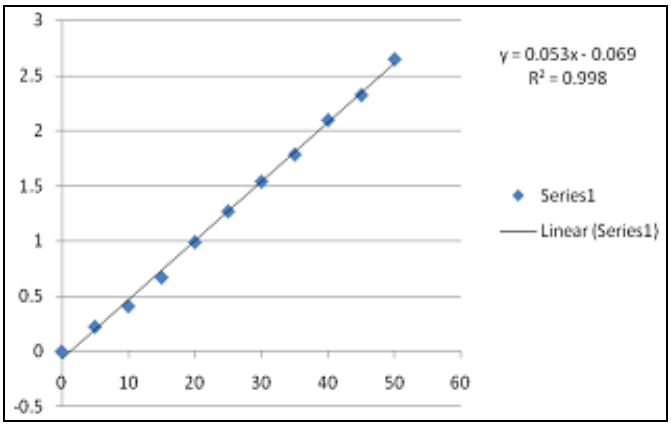


**FIG.1: CALIBRATION CURVE OF DICLOFENAC SODIUM AT 275nm**

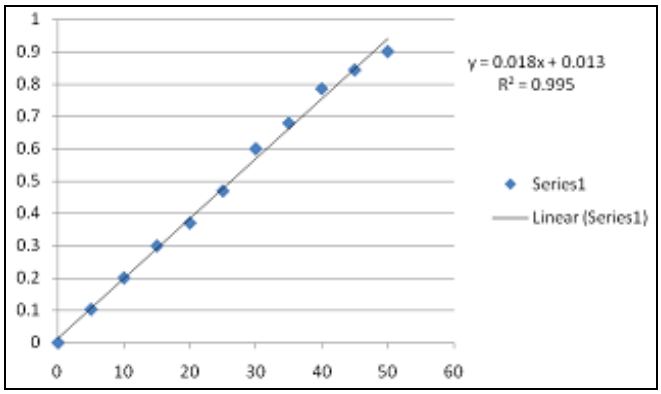


**FIG.2: CALIBRATION CURVE OF SERRATIOPEPTIDASE AT 264nm**

**TABLE 1: OPTICAL AND REGRESSION CHARACTERISTICS**

| Parameters | DC(275nm) | SER(264nm) |
|---|---|---|
| Slope | 0.053 | 0.018 |
| Intercept | 0.069 | 0.013 |
| Correlation coefficient | 0.998 | 0.995 |
| Linearity range (µg/ml) | 5-50 | 5-50 |
| LOD(µg/ml) | 0.247 | 0.150 |
| LOQ(µg/ml) | 0.748 | 0.455 |

**TABLE 2: ANALYSIS OF MIXED STANDARDS (BULK MIXTURE):**

| Conc. of drugs taken (µg/ml) | | Conc. of drugs found (µg/ml) | | *% Drug found | |
|---|---|---|---|---|---|
| SER | DC | SER | DC | SER | DC |
| 10 | 50 | 9.95 | 49.39 | 99.5% | 98.78% |

*Average of Five determinations

**TABLE 3: ANALYSIS OF TABLET FORMULATION**

| Label Claim (mg/ tablet) | | Amount found (mg/ tablet) | | % of Label Claim Estimated | |
|---|---|---|---|---|---|
| SER | DC | SER | DC | SER | DC |
| 10 | 50 | 9.20 | 48.50 | 92% | 97% |

*Average of Five determinations

## Precision: Repeatability:

**TABLE 4: REPEATABILITY DATA (STATISTICAL EVALUATION) BULK MIX**.

| Sr. No. | Drugs | * % Mean | *S.D | *% R.S.D. |
|---|---|---|---|---|
| 1 | SER | 97.66 | 0.201 | 0.205 |
| 2 | DC | 93.48 | 0.070 | 0.074 |

*Average of Five determinations

**TABLE 5: REPEATABILITY DATA (STATISTICAL EVALUATION) TAB MIX**

| Sr. No. | Drugs | * % Mean | *S.D | *% R.S.D. |
|---|---|---|---|---|
| 1 | SER | 94.96 | 0.0054 | 0.0057 |
| 2 | DC | 98.39 | 0.0109 | 0.0111 |

*Average of Five determinations

**TABLE 6: INTERMEDIATE PRECISION (INTER-DAY AND INTRA-DAY PRECISION):**

| DRUGS | Inter- Day | | | Intra-Day | | |
|---|---|---|---|---|---|---|
| | %Mean | SD | %R.S.D. | %Mean | SD | %R.S.D. |
| SER(BULK) | 95 | 0.207 | 0.217 | 95.9 | 0.125 | 0.130 |
| SER(TAB) | 92.44 | 0.0054 | 0.0058 | 93.06 | 0.0054 | 0.0058 |
| DC(BULK) | 93.42 | 0.070 | 0.074 | 93.48 | 0.070 | 0.074 |
| DC(TAB) | 98.49 | 0.4505 | 0.4574 | 98.4 | 0.0070 | 0.0071 |

* Average of Five determinations

**TABLE 7: ACCURACY: STATISTICAL VALIDATION OF RECOVERY STUDIES:**

| Level of % recovery | % Mean recovery* | | Standard deviation | | % R.S.D | |
|---|---|---|---|---|---|---|
| | SER | DC | SER | DC | SER | DC |
| 80 | 96.84 | 98.01 | 0.650 | 0.159 | 0.671 | 0.162 |
| 100 | 96.8 | 98.97 | 0.293 | 0.217 | 0.302 | 0.219 |
| 120 | 98.23 | 98.38 | 0.305 | 0.238 | 0.310 | 0.241 |

**TABLE 8: LINEARITY AND RANGE**

| DRUG | Diclofenac Sodium | | Serratiopeptidase | |
|---|---|---|---|---|
| Sr. No | Conc.(µg/ ml) | Absorbance at 275nm | Conc.(µg/ml) | Absorbance at 264nm |
| 1 | 5 | 0.225 | 5 | 0.103 |
| 2 | 10 | 0.412 | 10 | 0.200 |
| 3 | 15 | 0.672 | 15 | 0.299 |
| 4 | 20 | 0992 | 20 | 0.370 |
| 5 | 25 | 1.271 | 25 | 0.469 |
| 6 | 30 | 1.539 | 30 | 0.599 |
| 7 | 35 | 1.785 | 35 | 0.679 |
| 8 | 40 | 2.097 | 40 | 0.785 |
| 9 | 45 | 2.323 | 45 | 0.843 |
| 10 | 50 | 2.648 | 50 | 0.900 |

## Multicomponent Mode Method:
## Analysis of Mixed Standards (Bulk Mixture):

**TABLE 9: ANALYSIS OF MIXED STANDARDS (BULK MIXTURE)**

| Conc. of drugs taken (µg/ml) | | Conc. of drugs found (µg/ml) | | *% Drug found | |
|---|---|---|---|---|---|
| SER | DC | SER | DC | SER | DC |
| 10 | 50 | 10.02 | 50.03 | 100.2% | 100.6% |

*Average of Five determinations

**TABLE 10: ANALYSIS OF TABLET FORMULATION**

| Label Claim (mg/ tablet) | | Amount found (mg/ tablet) | | % of Label Claim Estimated | |
|---|---|---|---|---|---|
| SER | DC | SER | DC | SER | DC |
| 10 | 50 | 10.01 | 50.01 | 100.1% | 100.2 % |

*Average of Five determinations

## Precision: Repeatability

**TABLE 11: REPEATABILITY DATA (STATISTICAL EVALUATION) BULK MIX**.

| Sr. No. | Drugs | * % Mean | *S.D | *% R.S.D. |
|---|---|---|---|---|
| 1 | SER | 100.02 | 0.0044 | 0.0043 |
| 2 | DC | 100.04 | 0.0042 | 0.0041 |

*Average of Five determinations

**TABLE12: REPEATABILITY DATA (STATISTICAL EVALUATION) TAB MIX**

| Sr. No. | Drugs | * % Mean | *S.D | *% R.S.D. |
|---|---|---|---|---|
| 1 | SER | 100.01 | 0.0089 | 0.0088 |
| 2 | DC | 100.02 | 0.0044 | 0.0043 |

*Average of Five determinations

**TABLE 13: INTERMEDIATE PRECISION (INTER-DAY AND INTRA-DAY PRECISION):**

| DRUGS | Inter- Day | | | Intra-Day | | |
|---|---|---|---|---|---|---|
| | Mean | SD | %R.S.D. | Mean | SD | %R.S.D. |
| SER(BULK) | 100.01 | 0.0089 | 0.0088 | 100.02 | 0.0044 | 0.0043 |
| SER(TAB) | 100.02 | 0.0044 | 0.0043 | 100.01 | 0.0089 | 0.0088 |
| DC(BULK) | 100.02 | 0.0044 | 0.0043 | 100.02 | 0.0044 | 0.0043 |
| DC(TAB) | 100.04 | 0.0043 | 0.0042 | 100.4 | 0.0043 | 0.0042 |

*Average of Five determinations

## Accuracy:

**TABLE 14: VALIDATION OF RECOVERY STUDIES**

| Level of % recovery | % Mean recovery* | | Standard deviation | | % R.S.D | |
|---|---|---|---|---|---|---|
| | SER | DC | SER | DC | SER | DC |
| 80 | 100.01 | 100.01 | 0.0173 | 0.0152 | 0.0172 | 0.0151 |
| 100 | 99.99 | 100.01 | 0.0251 | 0.0115 | 0.0251 | 0.0114 |
| 120 | 100.01 | 100.02 | 0.0054 | 0.0057 | 0.0056 | 0.0056 |

*Average of three at each level of recovery

## RESULTS AND DISCUSSION:

Four simple, economic and validated UV-Spectrophotometric methods were developed for the simultaneous estimation of Diclofenac sodium and Serratiopeptidase in bulk and tablet formulation. The optimized parameters for UV-Spectrophotometer and the results of the validation are given in **Table 15.** The validation parameter studies suggest that the developed UV Spectrophotometric methods can be employed successfully for the estimation of SER and DC in both bulk and tablet Dosage form.

**TABLE 15: SPECTROPHOTOMETER AND THE RESULTS OF THE VALIDATION**

| Parameters | Simultaneous Equation Method | | Multi-Component Mode Method | |
|---|---|---|---|---|
| | SER | DC | SER | DC |
| Linearity range (µg/ml) | 5- 50 | 5- 50 | 5- 50 | 5- 50 |
| Correlation coefficient | 0.998 | 0.995 | 0.998 | 0.995 |
| LOD (µg/ml) | 0.150 | 0.247 | 0.150 | 0.247 |
| LOQ (µg/ml) | 0.455 | 0.748 | 0.455 | 0.748 |
| Intraday(%RSD)Bulk | 0.130 | 0.074 | 0.0043 | 0.0043 |
| Intraday(%RSD) tab | 0.0058 | 0.0071 | 0.0088 | 0.0042 |
| Inter-day(%RSD)bulk | 0.217 | 0.074 | 0.0088 | 0.0043 |
| Inter-day(%RSD)tab | 0.0058 | 0.4574 | 0.0043 | 0.0042 |
| Mean % recovery bulk | 97.66 | 93.48 | 100.02 | 100.04 |
| Mean % recovery tab | 94.96 | 98.39 | 100.01 | 100.02 |

**SUMMARY AND CONCLUSION:** The literature survey gives no reported UV visible spectrophotometric method for estimation of Diclofenac Sodium and Serratiopeptidase in bulk and Tablet dosage form. This work has made an attempt to develop two different UV visible spectrophotometric method for estimation of Diclofenac Sodium and Serratiopeptidase in bulk and Tablet dosage form. Two different UV visible spectrophotometric methods i.e. Simultaneous equation method, Multicomponent mode method were developed. The developed methods were validated for Accuracy, Precision (Repeatability, Intermediate precision and Reproducibility), Linearity, Range, Specificity, Limit of Detection (LOD), Limit of Quantitation (LOQ), Robustness, Ruggedness as per ICH Guidelines.

The proposed work concludes that, the developed two UV visible spectrophotometric methods i.e. Simultaneous equation method, and Multicomponent mode method are simple, accurate, rapid, sensitive, precise and economic. Hence these methods can be employed successfully for the estimation of Serratiopeptidase and Diclofenac sodium in both bulk and tablet dosage form.

We would like to thank Zydus cadila, PTC Thane and Analab fine chemicals for providing Diclofenac sodium and Serratiopeptidase A.P.I. gift sample.

## REFERENCES:

1. Indian pharmacopoeia, Vol-II, 1996, Ministry of Health & family welfare, Govt. of India, Controller of publications, New delhi, 1158.
2. Indian Pharmacopoeia 2007. Published by the Government of India, Ministry of Health and Family Welfare, The Indian Pharmacopoeia commission, New Delhi Volume I & II;632.
3. Ashok R. Parmar, Dharmishtha N. Bhakhar, Dolita K. Shah, Dr. Shailesh Koradiya and Vasant D. Khasia, Spectrometric Determination of Aceclofenac and Serratiopeptidase in Tablet Dosage Form by Area under Curve Method Journal of Pharmacy Research 2012,5(8),3981-3984.
4. Ashok R. Parmar, Dharmishtha N. Bhakhar, Dolita K. Shah and Kinjal V. Vekariya Simultaneous estimation of Aceclofenac and Serratiopeptidase in Tablet Dosage Form by Absorbance Ratio Method using Visible Spectrophotometry Pelagia Research Library Der Pharmacia Sinica, 2012, 3 (3):321-326
5. Swartz E. Michael, Krull S. Ira, Analytical Method Development & Validation; 53-67, 73.

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