

**METHOD 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

A Second order derivative spectroscopy and RP-HPLC methods were developed and validated for simultaneous estimation of Diclofenac sodium(DFS) and Serratiopeptidase(SPD) in Tablet dosage form. Accurate and precised UV spectrophotometric method with good sensitivity has been developed for simultaneous estimation of DFS and SPD. The method employed Second order derivative method 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 RP-HPLC method has shown adequate separation of DFS and SPD in Tablet dosage form. The separation was achieved on a Enable C18 (250 mm - 4.6 mm, 5 µm particle size) with an isocratic system of Acetonitrile: Phosphate buffer in the ratio of 55:45 v/v. The mobile phase at a flow rate of 1.5 ml/min, Injection volume 20µl and wavelength of detection used was 277nm. The retention time for DFS and SPD was obtained as 1.277 min and 6.317 min respectively. The linearity of the proposed method was investigated in the range of 5-25 µg/ml and 25-125 µg/ml for DFS and SPD respectively. 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.

Key words: Diclofenac sodium, Serratiopeptidase, Second order derivative method, RP-HPLC, Validation.