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# Derivative Spectrophotometric Method For Simultaneous Estimation Of Nimesulide And Serratiopeptidase From Tablet Dosage Form

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## ABSTRACT

The first derivative spectrophotometric method for simultaneous estimation of nimesulide(NL) and serratiopeptidase(SP) in two component solid dosage forms has been developed. The method utilizes phosphate buffer pH 7.4 as a solvent. In this solvent system, the first derivative spectrum of NL shows the equal absorbance at 223nm and 250 nm and for the estimation of SP, the difference between the absorbance at 223nm and 250nm was used. The spectrum of SP shows the zero absorbance at 360.5nm, this zero crossing point was used for the estimation of NL. The linearity ranges for NL and SP were 10-90 μg/ml and 2-90 μg/ml, respectively. 

## KEYWORDS

Nimesulide;
Serratiopeptidase
Simultaneous estimation.

## INTRODUCTION

Nimesulide(NL) and serratiopeptidase(SP) are the drugs of choice to relief pain and inflammatory conditions without any considerable abuse liability. The fixed dosage combination of these drugs widely prescribed nowadays because of high therapeutic threshold, fast recovery and more patient compliance[1].

Literature survey reveled that SP has been determined by HPLC[2] and NL by colorimetric[3-4], spectrophotometric[5-8], HPLC[9-11], HPTLC[12] and GC[13] methods. There is no spectrophotometric method has been reported for the simultaneous estimation of both the components in a combined dosage form. The objective of this investigation was to develop simple, accurate and economical procedures for simultaneous estimation of NL and SP from a tablet dosage form.

## MATERIALS AND METHOD

### Instrument, reagents and chemicals

A dual-beam Shimadzu UV-visible spectrophotometer 1700 Pharmaspec was used for the analysis.

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Freshly prepared phosphate buffer pH 7.4 in doubled distilled water was used as a solvent. Gift samples of NL and SP were procured from Zim Labs. Ltd., Nagpur and Advanced Technologies Ltd., Nashik, respectively.

### Preparation of standard stock solution

Standard stock(1 mg/ml) and working solutions(0.1 mg/ml) of NL and SP were prepared in phosphate buffer pH 7.4.

### Spectral and linearity characteristics of NL and SP

For spectral characteristics the aliquot portions equivalent to 30µg/ml of NL and SP were accurately transferred into two 10ml volumetric flasks separately and the volume was completed with phosphate buffer pH 7.4. The first derivative spectra were recorded(Figure 1 and 2).

Aliquot portions(0.2, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10ml) from the 0.1 mg/ml NL and SP working solutions were accurately transferred to 10ml volumetric flasks, the volume was made up with buffer pH 7.4. The first derivative absorption spectrum[14] of all the solutions was recorded between 200-400 nm. For the NL, the absorbance of solutions was measured at 360.5nm where, the absorbance of SP is zero. While for the SP, the absorbance of solutions was measured at 223nm and 250nm where, absorbance of NL is equal and difference of absorbance was zero.

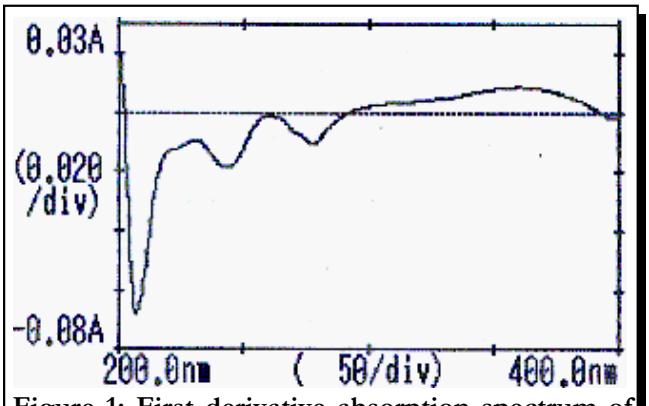


**Figure 1: First derivative absorption spectrum of nimesulide, concentration is 30-µg/ml**

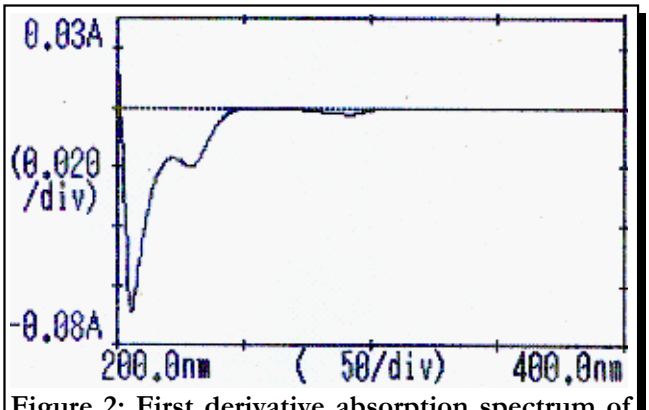


**Figure 2: First derivative absorption spectrum of serratiopeptidase concentration is 30-µg/ml**

### Application of the proposed procedure for the determination of NL and SP in tablets and laboratory prepared mixtures

Twenty tablets were weighed and average weight was determined. The blend was taken and was crushed to fine powder. The powder equivalent to 100mg of NL was transferred into 100ml volumetric flask. The powder was dissolved in 75ml of buffer pH 7.4 by intermittent shaking and volume was made up to 100ml with the same solvent. The solution was then filtered through a Whatmann filter paper (No. 41). The solution was diluted with buffer pH 7.4 to obtain 50µg/ml of NL and 5µg/ml of SP. This sample was scanned over the range of 200nm to 400nm in spectrum mode. Then the spectrum has

**TABLE 1: Determination of NL and SP in tablet using the proposed methods**

| | Recovery (%) ±S.D. (n=6) | | RSD (%) | |
|---|---|---|---|---|
| | NL | SP | NL | SP |
| Tablet | 100.33±0.272 | 100.00±0.238 | 0.271 | 0.238 |

S.D.: Standard deviation, RSD: Relative standard deviation

**TABLE 2: Results of the analysis of NL and SP in the laboratory prepared mixtures using the proposed methods**

| Sample no. | Concentration (µg/ml) | | Recovery (%) | |
|---|---|---|---|---|
| | NL | SP | NL | SP |
| 1 | 30 | 00 | 100.23 | -- |
| 2 | 25 | 05 | 100.12 | 99.45 |
| 3 | 20 | 10 | 100.21 | 100.8 |
| 4 | 15 | 15 | 100.12 | 99.89 |
| 5 | 10 | 20 | 100.32 | 100.28 |
| 6 | 05 | 25 | 100.16 | 100.31 |
| 7 | 00 | 30 | -- | 99.99 |
| Mean | | | 100.19 | 100.25 |
| Standard deviation | | | 0.085 | 0.355 |

**TABLE 3: Results of the application of the standard addition technique to the simultaneous determination of NL and SP in tablet by the proposed method. (n=6)**

| Claimed amount taken(µg/ml) | | Standard added (µg/ml) | | Recovery of added standard (%)[a] ± S.D. | |
|---|---|---|---|---|---|
| NL | SP | NL | SP | NL | SP |
| 50 | 5 | 25 | 2.5 | 100.14±1.018 | 99.98±0.118 |
| 50 | 5 | 50 | 5 | 100.15±1.024 | 100.2±0.001 |
| 50 | 5 | 62.5 | 6.25 | 100.16±1.102 | 100.3±0.004 |
| Mean | | | | 100.15±1.042 | 100.16±0.357 |

a: Mean, S. D.: Standard deviation.

been changed to first derivative spectra. For the estimation of both these drugs the absorbance of tablet solution was measured at 223, 250 and 360.5nm from first derivative spectra. The analysis procedure was repeated six times(TABLE 1). The selectivity of the proposed procedures was examined by determining the recovery of the two drugs in laboratory-prepared mixtures containing different ratio of the two drugs(TABLE 2) and by standard addition method(TABLE 3).

## RESULTS AND DISCUSSION

The first derivative spectra (figure 1 and 2) of NL and SP, shows that, the absorbance of SP is zero at 360.5nm whereas, the absorbance of NL has same absorbance at 223 and 250nm, respectively. Therefore, these wavelengths were selected for the estimation of NL and SP from the tablet solution. For the measurement of NL and SP, the absorbances of solutions were measured at 360.5nm(zero absorbance of SP) and at 223 and 250nm(equal absorbance of NL). After putting the values of absorbance in the following equation, the concentration of NL and SP in µg/ml was calculated.

$C_{NL} = (A_1+0.0004)/ 0.0003$

$C_{SP} = (0.0004- \Delta A_2)/ 0.0003$

Where, $A_1$ is the absorbance of tablet sample solution at 360.5nm and $\Delta A_2$ is the difference of 223 and 250nm, respectively. $C_{NL}$ and $C_{SP}$ are the concentration of NL and SP in µg/ ml.

### Validation of method[15]

The method was validated with respects to linearity, limit of detection(LOD), limit of quantification (LOQ), precision, accuracy and selectivity/sensitivity. The results obtained are summarized in TABLE 4 and 5, respectively.

**TABLE 4: Data for calibration graph for determination of NL and SP**

| Parameters | NL | SP |
|---|---|---|
| Slope | 0.003 | -0.0003 |
| Intercept | -0.0004 | 0.0004 |
| Correlation coefficient | 0.9987 | 0.9983 |
| Linearity range(µg/ml) | 10-90 | 2-90 |
| LOD(µg/ml) | 1.37 | 1.41 |
| LOQ(µg/ ml) | 4.15 | 4.02 |

**TABLE 5: Precision and accuracy of method developed for analysis of formulation(n=6)**

| | Amount found (Mean %± S. D.) | | Accuracy, Bias (%) | | Precision, RSD (%) | |
|---|---|---|---|---|---|---|
| | NL | SP | NL | SP | NL | SP |
| Inter day tablet | 100.33±0.271 | 100.01±0.238 | 0.33 | 0.01 | 0.27 | 0.24 |
| Intra day tablet | 100.6 ±0.215 | 100.5±0.212 | 0.6 | 0.5 | 0.21 | 0.021 |

S.D.: Standard deviation, % Bias=[100(found- label claim)/label claim], RSD: Relative standard deviation

## CONCLUSION

The proposed method was found to be simple, accurate, economical and rapid for routine simultaneous estimation of two drugs. The method was found to be economical, as it requires only buffer pH 7.4 as a solvent. The results obtained for tablets, laboratory mixtures and recovery studies are summarized in TABLE 1, 2 and 3, respectively. The results of validation parameters shown in TABLE 4 and 5 are satisfactory in level indicates the accuracy of proposed method for estimation of NL and SP.

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