Simultaneous Estimation of Valdecoxib and Tizanidine Hydrochloride in Tablets by RP-HPLC

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A simple, fast, precise and accurate RP-HPLC method was developed for the simultaneous estimation of valdecoxib and tizanidine hydrochloride in tablet formulations. The separation was achieved by C18 Intersil column and acetonitrile: 0.02 M phosphate buffer (pH 3.5) (60:40 v/v) as mobile phase, at a flow rate of 1.5 ml/min. Detection was carried out at 240 nm. The retention time of valdecoxib and tizanidine hydrochloride was found to be 4.21 and 2.16 min respectively. The validation of the proposed method was also carried out for linearity, accuracy and precision. The linear dynamic range for valdecoxib and tizanidine hydrochloride was 0-100 µg/ml and 0-20 µg/ml respectively. The mean percentage recoveries obtained for valdecoxib and tizanidine hydrochloride were 99.10 and 100.19% respectively. The developed method was found to be accurate, precise, selective and rapid, and it can also be used for routine quality control analysis of these drugs in combination tablets.

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Valdecoxib (VALD), 4-(5-methyl-3-phenyl-4-isoxazolyl) benzenesulfonamide, is the newest specific cyclooxygenase-2 (cox-2) inhibitor. It is active at low dose and has less gastric toxicity. It is mainly used for the treatment of osteoarthritis, rheumatoid arthritis and dysmenorrhea. Tizanidine hydrochloride (TIZA), 5-chloro-4-(2-imidazolin-2-yl-amino)-2,1,3-benzothiadiazole, is used as a skeletal muscle relaxant. A combination of these drugs, TIZA (2 mg) and VALD (20 mg), in tablet formulation is available commercially (Valeron MR, Shince Pharmaceutical, Chennai). Literature survey revealed that HPLC methods are reported for the determination of VALD in only human plasma. An HPTLC method for estimation of TIZA and also RP-HPLC methods for simultaneous estimation of TIZA in combination with nimesulide and also in combination with rofecoxib and another combination with dextropropoxyphene hydrochloride and acetaminophen in capsule have been reported. The present work describes the development of a simple, precise and accurate reverse phase HPLC method for simultaneous estimation of VALD and TIZA.

The drug samples of VALD and TIZA were obtained as gift samples from Aeon Therapeutics, Chennai; and Bakul Pharma, Ankleshwar, respectively. HPLC grade acetonitrile, potassium dihydrogen phosphate AR and O-phosphoric acid AR were supplied by Merck Co., Mumbai. HPLC grade water was supplied by Loba Chemicals, Mumbai. The liquid chromatographic system used was equipped with Jasco PU 1580 intelligent pump, variable wavelength UV/Vis (Jasco UV 1575) detector and precision loop injector (Rheodyne 20 µl). The column C18 Intersil (250 × 4.6 mm i.d., particle size 10 µ) was used as a stationary phase.

The mobile phase used was a mixture of acetonitrile and 0.02 M phosphate buffer (adjusted to pH 3.5 using orthophosphoric acid) in the ratio of 60:40 v/v; it was filtered before use through Whatman filter paper no. 41. The elution was carried out isocratically at the flow rate of 1.5 ml/min. Detection was carried out at 240 nm at ambient temperature.

Standard stock solutions of VALD and TIZA (1 mg/ml) were prepared in mobile phase. To study the linearity range of the drugs, serial dilutions were made from standard stock solutions in the range of 0-100 µg/ml of VALD and 0-20 µg/ml of TIZA. A graph was plotted as concentration of drugs versus peak area response, and it was found to be linear for both the drugs. From the standard stock solutions, mixed standard solution was prepared containing 20 µg/ml of VALD and 2 µg/ml of TIZA. The system suitability test was performed by collecting data from five replicate injections of mixed standard drugs solutions.

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of powder equivalent to 100 mg of VALD and 10 mg of TIZA was taken in 100 ml volumetric flask and dissolved in mobile phase. Volume was made up to mark with mobile phase. The solution was filtered through Whatman filter paper no. 41. The aliquot portion of the filtrate was further diluted to get final concentration of 20 µg/ml of VALD and 2 µg/ml of TIZA. Twenty microlitres of the test and standard solutions were injected separately and chromatogram was recorded for the same, and the amounts of the drugs were calculated.

The present study was carried out to develop a simple and rapid HPLC method for estimation of VALD and TIZA in tablets using C18 column. The mobile phase was optimized with acetonitrile and 0.02 M phosphate buffer (pH 3.5) in the ratio of 60:40 v/v, with flow rate of 1.5 ml/min. UV detection was carried out at 240 nm and run time 10 min. The retention time of VALD and TIZA was found to be 4.21 and 2.16 min respectively. A typical chromatogram of the test solution is shown in fig.1. The capacity factor (k') of VALD and TIZA was found to be 3.21 and 1.16 respectively, and the tailing factor was less than 2.0. The proposed method was validated (Table 1). Accuracy of

![Fig. 1: Typical chromatogram of the sample solution](https://example.com/figure1.png)

TIZA stands for tizanidine and VALD stands for valdecoxib.
Spectrophotometric Determination of Gatifloxacin in Pharmaceutical Formulations and Biological Samples

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Three new simple and sensitive spectrophotometric methods in ultraviolet region have been developed for the determination of gatifloxacin in bulk drug, pharmaceutical preparations and biological samples. Gatifloxacin exhibited maximum absorbance at 289 nm (method A) with apparent molar absorptivity of $1.23 \times 10^4$ l/mol×cm when dissolved in sodium hydroxide; and maximum absorbance at 292 nm (method B) with apparent molar absorptivity of $0.3488$ l/mol×cm.

The method was calculated by recovery studies at three levels by standard addition method. The average recovery of VALD and TIZA was 99.10 and 100.19% respectively. Precision of the method was established by replicate analysis of the analyte (five times) using the proposed method. The low values of SD and RSD indicated that the proposed method has good precision. Linearity was determined for VALD in the range of 0-100 µg/ml; and for TIZA, 0-20 µg/ml. The correlation coefficient ($'r^2'$) values for both the drugs were >0.999. Ruggedness study (inter-day, intra-day) signifies the reproducibility of the method.

Based on the validation study data, it can be concluded that the proposed method is accurate and precise for the analysis of both the drugs. No interference was found from excipients used in tablet formulation and hence the method is suitable for analysis of tablet formulation. The method is simple and has a total runtime of 10 min, which makes it especially suitable for routine quality control analysis work.

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REFERENCES


TABLE 1: VALIDATION PARAMETERS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Valdecoxib</th>
<th>Tizanidine HCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/ml)</td>
<td>0-100</td>
<td>0-20</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.9991</td>
<td>0.9995</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.3301</td>
<td>0.3488</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>99.10±0.14</td>
<td>100.19±0.72</td>
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<tr>
<td>Intra day (n=3) %</td>
<td>99.49±0.38</td>
<td>99.36±0.06</td>
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<tr>
<td>Inter day (n=3) %</td>
<td>99.52±0.20</td>
<td>99.32±0.18</td>
</tr>
</tbody>
</table>

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