Determination of Diflunisal in Tablets Using Derivative UV Spectrophotometric Methods

Filiz SAYIN*, Sedef KIR*

Summary
In this study, derivative UV spectrophotometric methods were developed for the analysis of diflunisal, which is used as a non-steroidal anti-inflammatory drug. Analysis wavelengths of diflunisal for the first and second order derivative UV spectrophotometric methods were obtained as 235.6 nm and 228.8 nm, respectively. The linearity ranges were found as 1.0-25.0 µg mL⁻¹ for the first order and 1.0-20.0 µg mL⁻¹ for the second order derivative UV spectrophotometric method. The detection limit was 0.1 µg mL⁻¹ for both developed methods. Derivative UV spectrophotometric methods were also applied to the synthetic samples and tablets containing diflunisal. The results of tablet analysis, which were obtained by derivative UV spectrophotometric methods, were compared with spectrofluorimetric method in the literature. No statistically significant difference was found between diflunisal concentrations obtained from the three methods. The mean percentage recoveries and mean relative standard deviations were found to be lower than 2% by statistical evaluation.

Key Words: Diflunisal, derivative UV spectrophotometry, tablet analysis, spectrofluorimetry.

INTRODUCTION

Diflunisal [5- (2',4'-difluorophenyl) salicylic acid] is a synthetic analog of salicylic acid, with analgesic and anti-inflammatory activity 1,2.

* Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry 06100 Ankara - TURKEY
° Corresponding author e-mail: filizs@hacettepe.edu.tr
Comparison of the pharmacological profile of diflunisal with that of some well-known anti-inflammatory agents such as aspirin, ibuprofen and indomethacin has shown that diflunisal is more potent and less toxic than these drugs. Several methods were described for the determination of diflunisal, such as difference spectrophotometry, spectrofluorimetry, nuclear magnetic resonance spectroscopy, chromatography, immunoassay and electrochemical methods.

No derivative spectrophotometric study has been found in the literature for diflunisal. The aim of this work was to develop a method for the determination of diflunisal by UV spectrophotometry and to apply this method to the pharmaceutical preparations.

An analytical method has not been presented for the analysis of diflunisal in the presence of tablet excipients (sunset yellow: E110 and titanium dioxide: TiO₂) in a pharmaceutical dosage form. Thus, for assay of diflunisal, simple and reliable methods were developed.

In this paper, two methods based on derivative UV spectrophotometry are proposed for the quantitation of diflunisal in synthetic and commercial tablets.

The results obtained from derivative UV spectrophotometric methods were statistically compared with spectrofluorimetric method in the literature.

**MATERIALS and METHODS**

**Materials**

**Apparatus**

A Shimadzu (UV-VIS) spectrophotometer UV-160-A with 1.000 cm quartz cuvette was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: fast (2400 nm/min); sampling interval: 0.2 nm; cycle time: 60 s; derivative mode: 1D (first order derivative, dA/dλ) and 2D (second order derivative, d²A/dλ²); band width (λ): for 1D, 17.5 nm (N=5) and for 2D, 24.5 nm (N=7); spectral slit width: 2 nm.

Spectrofluorimetric measurements were performed on Shimadzu RF-5301 PC spectrofluorophotometer equipped with 1-cm quartz cuvette and a 150-W Xenon lamp. The condition selected: scan range: 220-410 nm for excitation (EX) and 300-500 nm for emission (EM); wavelength: λ<sub>EX</sub> 260 nm (λ<sub>EM</sub>: 425 nm) and λ<sub>EM</sub>: 425 nm (λ<sub>EX</sub>: 260 nm); scan speed: fast; slit width: 1.5 nm for EX and 5.0 nm for EM.

**Reagents and Solutions**

All experiments were performed with analytical grade chemicals and double distilled deionized water. Standard diflunisal (Sigma) was used without further purification. DOLPHIN® 500 (Adilna-Sanovel Drug, Industry) tablets, labelled to contain 500 mg diflunisal, were obtained from a local pharmacy.

**Methods**

**Preparation of Standard Solutions**

Stock solutions of diflunisal and E110 (1000µg mL⁻¹) were prepared in methanol. Working standard solutions were obtained by diluting the stock solutions with concentrations ranging from 0.05 µg mL⁻¹ to 30 µg mL⁻¹ for diflunisal and from 0.05 µg mL⁻¹ to 40 µg mL⁻¹ for E110 in methanol. Working standard solutions were prepared daily.

**Preparation of Synthetic Tablet Sample Solutions**

500 mg of diflunisal and other excipients (E110: 0.2 mg and TiO₂: 6.26 mg) were weighed and transferred into a 100 mL calibrated flask. It was then mixed with 50 mL of methanol. The mixture was mechani-
cally shaken for 15 min and diluted to the volume with methanol. Then, a suitable µL portion of the clear solution was used for the analysis.

**Preparation of Tablet Sample Solutions**

The average mass of 10 tablets was determined. The content of tablets was powdered and the amounts corresponding to 500 mg of diflunisal were weighed. Subsequent preparations of tablet samples were treated as described above for the synthetic tablet sample solutions.

**Procedures**

10 µL aliquots were taken from each of the synthetic tablet sample solutions, and tablet sample solutions were prepared in methanol, transferred into 5 mL calibrated flask, and completed to the volume with methanol. Then, first order derivative-UV (1D-UV) and second order derivative-UV (2D-UV) spectra of prepared solutions were recorded against methanolic solution as blank. Quantitation of diflunisal in the sample solutions was calculated from the calibration graphs and statistically evaluated.

**RESULTS and DISCUSSION**

**Derivative UV Spectrophotometric Methods**

In our study, diflunisal was analyzed using 1D-UV and 2D-UV spectrophotometric methods. Derivative UV spectrophotometry offers greater selectivity than UV spectrophotometry because it decreases spectral overlap and allows better resolution.

At the beginning, the absorption (zero order) UV spectra of diflunisal and E110 over the range 200 – 400 nm in methanol were recorded (Fig. 1a and 1b), respectively. As seen in Figure 1, the spectral bands of diflunisal and E110 were overlapped. Thus, conventional UV spectrophotometry cannot be used for determination of diflunisal presence of E110. However, when 1D-UV and 2D-UV spectra were recorded, sharp bands with high amplitudes (Figs. 2 and 3 respectively) were obtained, which might permit more selective identification and determination of diflunisal and E110 in the standard.
For spectrophotometric methods, the nature of the solution, degree of derivative, range of wavelength, "N" value and pathlength were optimized and are given in the experimental section.

In the first method, 1D-UV spectrum (Fig. 2a) permitted the determination of diflunisal at 235.6 nm (zero crossing of E110) without any interference from E110. Additionally, E110 can be determined at 247.4 nm (zero crossing of diflunisal) (Fig. 2b). Thus, 235.6 nm was selected as a wavelength for the quantitative studies of diflunisal by 1D-UV spectrophotometric method. The calibration curves of diflunisal and E110 at 235.6 nm and 247.4 nm, respectively, are given in Figure 4.

For the second method, analysis wavelengths were selected as 228.8 nm for diflunisal and 234.4 nm for E110 (zero crossing technique) from 2D-UV spectrum (Fig. 3), and the calibration curves of diflunisal and E110 were performed at these wavelengths (Fig. 5).

**Analytical data**

The linear range of concentrations for the analysis of diflunisal was found to be 1.0-25.0 µg mL⁻¹ and 1.0-20.0 µg mL⁻¹ for 1D-UV and 2D-UV spectrophotometric methods, respectively. The calibration curves, obtained by the recommended procedure, were linear over the range 1.0-40.0 µg mL⁻¹ and 1.0-35.0 µg mL⁻¹ of E110 for 1D-UV and 2D-UV spectrophotometric methods, respectively. The linearity was checked by preparing standard solutions at 11 different concentrations. The linearity of the calibration graphs and conformity of the 1D-UV and 2D-UV measurements of the proposed methods to Beer’s law were proven by the high values of the correlation coefficient (r) of the regression equations.

The limit of detection (LOD) is the lowest concentration that can be distinguished from noise level (signal/noise, S/N=3). LOD for diflunisal and E110 was found to be 0.10 µg mL⁻¹ for 1D-UV and 2D-UV spectrophotometric methods. The limit of quantitation (LOQ) is described as the lowest concentration which can be determined with acceptable accuracy and precision for the substance being analyzed within the limits according to the specified conditions of the developed methods. LOQ for diflunisal and E110 was determined as 1.00 µg mL⁻¹ for both methods. LOD and LOQ, linearity ranges, equation of calibration curves, correlation coefficient and standard error of correlation coefficient values for the methods are given in Table 1.

**Table 1**: Determined Parameters for the Calibration Curves of Diflunisal Obtained from the Developed Methods (n = 11)

<table>
<thead>
<tr>
<th>Methods</th>
<th>LOD (µg mL⁻¹)</th>
<th>LOQ (µg mL⁻¹)</th>
<th>Linearity range (µg mL⁻¹)</th>
<th>Regression equation (y = a + bx)</th>
<th>r</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D-UV</td>
<td>0.10</td>
<td>1.0</td>
<td>1.0-25.0</td>
<td>y (D) = 0.005 - 0.028 x (C)</td>
<td>0.9993</td>
<td>0.01</td>
</tr>
<tr>
<td>2D-UV</td>
<td>0.10</td>
<td>1.0</td>
<td>1.0-20.0</td>
<td>y (D) = 0.040 - 0.068 x (C)</td>
<td>0.9995</td>
<td>0.01</td>
</tr>
</tbody>
</table>

LOD: Limit of detection, LOQ: Limit of quantitation, a: Intercept, b: Slope, x: Concentration of diflunisal, y: Amplitude of 1D-UV spectrum and 2D-UV spectrum, r : Correlation coefficient, SE: Standard error of correlation coefficient

The utility of these methods was verified by means of a recovery assay in the synthetic tablet samples. Synthetic tablet samples containing 500 mg diflunisal...
sal, 0.2 mg E110 and 6.26 mg TiO₂ were prepared and processed according to the proposed methods. Recoveries were determined by comparison with the corresponding standard solutions. The mean percentage recoveries for 1D-UV and 2D-UV spectrophotometric methods were found to be 100.00% and 99.94%, respectively (Table 2). Therefore, the best accuracy was obtained by assay of the developed methods.

Table 2. Precision and Accuracy Studies for the Determination of Diflunisal Using the Developed Methods

<table>
<thead>
<tr>
<th>Methods</th>
<th>Sample no</th>
<th>Nominal value of diflunisal (mg)</th>
<th>Found value of diflunisal (x mg ± SD)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D-UV</td>
<td>1</td>
<td>500.0</td>
<td>500.10 ± 1.34</td>
<td>0.27</td>
<td>100.02</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>500.0</td>
<td>499.90 ± 1.23</td>
<td>0.25</td>
<td>99.95</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>500.0</td>
<td>500.10 ± 1.50</td>
<td>0.30</td>
<td>100.04</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>500.0</td>
<td>500.15 ± 1.32</td>
<td>0.26</td>
<td>100.03</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>500.0</td>
<td>500.10 ± 1.24</td>
<td>0.25</td>
<td>100.02</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>500.0</td>
<td>499.50 ± 1.39</td>
<td>0.28</td>
<td>99.90</td>
</tr>
<tr>
<td>2D-UV</td>
<td>1</td>
<td>500.0</td>
<td>500.10 ± 1.24</td>
<td>0.25</td>
<td>100.02</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>500.0</td>
<td>499.90 ± 1.12</td>
<td>0.22</td>
<td>99.78</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>500.0</td>
<td>499.25 ± 1.54</td>
<td>0.31</td>
<td>99.85</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>500.0</td>
<td>500.10 ± 1.34</td>
<td>0.27</td>
<td>100.02</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>500.0</td>
<td>499.50 ± 1.09</td>
<td>0.22</td>
<td>99.90</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>500.0</td>
<td>500.20 ± 1.47</td>
<td>0.29</td>
<td>100.04</td>
</tr>
</tbody>
</table>

(x (mg) ± SD): Mean ± standard deviation for five determinations, RSD: Relative standard deviation.

The precision and reproducibility of these developed methods for diflunisal were determined in five replicate analyses on a synthetic tablet sample (Table 2). The mean relative standard deviations were found to be 0.27% and 0.26% for 1D-UV and 2D-UV spectrophotometric methods, respectively. The relative standard deviations were found to be less than 2%, indicating precision of the two developed methods.

The specificity of the two methods was tested by examining the possible interference of tablet excipients in the assay procedure. Results of synthetic tablet studies showed that the possible interference related to additive absorption of the excipient E110 in UV was prevented by these derivative UV spectrophotometric methods. Thus, diflunisal was analyzed in tablet formulation using the developed methods. The stability of the standard and sample solutions was checked by analyzing these solutions stored in the dark at +4°C for one month and by heating and adding 0.1 N HCl solution for acidic conditions and 0.1 N NaOH solution for alkaline conditions. The results demonstrated that the working standard solution as well as the sample solution is stable for at least one month. During the stability studies no additional peaks developed and no changes in the spectrophotometric pattern were observed in either of the solutions. However, the spectrum of diflunisal was not observed in the 0.1 N HCl and 0.1 N NaOH solutions.

The spectrofluorimetric method was used as the comparative method, and it was applied to the assay of diflunisal in standards and tablets. Spectrofluorimetric spectrum of diflunisal is given in Fig. 6 ($\lambda_{EX} = 260$ nm, $\lambda_{EM} = 425$ nm).

Quantitative analysis of diflunisal in tablets in developed and comparative methods was performed using calibration curves.

The statistical comparison of the three methods, 1D-UV spectrophotometric, 2D-UV spectrophotometric and spectrofluorimetry, is given in Table 3. This comparison has been performed by variance analy-
sis (α=0.05; n=15), and no significant difference between the proposed methods and comparative method was found with respect to precision and accuracy, i.e. the calculated F value (F_C) did not exceed the theoretical F value (F_T).

**Table 3:** Statistical Evaluation and Comparison of Obtained Data from Developed Methods (500 mg diflunisal in one tablet of DOL-PHIN® 500)

<table>
<thead>
<tr>
<th>Statistical values</th>
<th>1D-UV method</th>
<th>2D-UV method</th>
<th>Spectrofluorimetric method</th>
<th>F values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>F_H = 6.89 x 10^{-3}</td>
</tr>
<tr>
<td>± SD</td>
<td>500.59 ± 0.38</td>
<td>500.74 ± 0.54</td>
<td>501.49 ± 0.37</td>
<td>FT = 3.34</td>
</tr>
<tr>
<td>RSD</td>
<td>0.28%</td>
<td>0.29%</td>
<td>0.29%</td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>500.18 - 501.80</td>
<td>499.59 - 501.90</td>
<td>500.70 - 502.74</td>
<td></td>
</tr>
</tbody>
</table>

n: Number of sample, \(\bar{x}\): Mean, SD: Standard deviation, RSD: Relative standard deviation, CI: Confidence interval (α= 0.05), FH: Calculated F value, FT: Theoretical F value (α=0.05)

**CONCLUSION**

The newly developed methods were validated by evaluation of the validation parameters. The methods developed in this study are satisfactorily sensitive, accurate, precise and reproducible for determination of diflunisal in tablet formulations even in the presence of excipient E110.

In view of the results of the present study, 1D-UV and 2D-UV spectrophotometric methods are proposed as suitable tools for the direct determination of diflunisal in pharmaceutical preparations as a tablet. In spite of the comparative method showing more sensitive than the proposed methods, variance analysis showed no significant differences between the performance of the proposed and comparative methods with respect to accuracy and precision.

Spectrofluorimetric measurements in the given comparative method were obtained by recording the difference in absorbance values of alkaline solutions against acidic solutions, but this was not done in the proposed methods. The linear concentration range of the newly developed methods was observed wider than the comparative method. In addition, the proposed methods are cheaper and simpler. Thus, selective derivative UV spectrophotometric methods are applicable for the quality control and routine analysis of diflunisal and may also be proposed for determination of diflunisal from biological fluids.

**REFERENCES**